THE AMERICAN NATURALIST

Vol. XCI September-October, 1957 No. 860

A STUDY OF PRIMATE CHROMOSOME COMPLEMENTS*

ERNEST H. Y. CHU AND NORMAN H. GILES

Department of Botany, Josiah Willard Gibbs Research Laboratories,
Yale University, New Haven, Connecticut

Recent rapid expansion of poliomyelitis research has resulted in an extensive use of monkey tissue in culture, thus making available otherwise rare materials for cytological and physiological studies. Furthermore, the advantages of studying mammalian chromosomes in tissue culture have been clearly pointed out in a recent account by Hsu (1952). His hypotonic pretreatment method for spreading chromosomes (Hsu and Pomerat 1953), in agreement with independent observations of Hughes (1952), gives excellent preparations suitable for critical karyological descriptions and comparisons.

The karyotypes of relatively few Primates have been determined by these modern cytological techniques now available. The need for such studies has been clearly demonstrated by the recent results of Tjio and Levan (1956) whose use of tissue culture preparations indicated that the somatic chromosome number of man is 46 rather than 48, a result which has been confirmed by the studies of Ford and Hamerton (1956).

The present report is concerned with descriptions of the chromosome complements of nine species in one family of Old World monkeys, the Cercopithecidae. These studies indicate a notable variation in chromosome number and morphology in this group of related species. It is anticipated that these and similar additional studies will help to elucidate evolutionary mechanisms and relationships in this family of Primates. Certain results of this work have been previously summarized (Chu and Giles, 1956).

METHODS

Tissue Culture. Cultures of kidney epithelial cells were prepared from monkeys by the method of Youngner (1954). The medium used consisted of 0.5% lactalbumin enzymatic hydrolysate, 2.0% calf serum, and 97.5% Hanks'

*This research has been supported in part under a contract, AT(30-1)-1908, with the Atomic Energy Commission.

balanced salt solution. Cells were grown on the glass surfaces of ordinary 3 oz. prescription bottles or on removable 22 x 11 mm cover slips in depression test tubes (Leighton tubes).

Cytological Preparations. After two days incubation at 35° C, cells were sampled daily for cytological examination. Single cells can be dissociated from the glass surface of bottles by incubation at 35° C for 10 minutes with 0.2% trypsin or 0.02% versene (ethylene dinitrilotetraacetic acid, disodium salt) solutions. The culture bottles selected was first rinsed briefly with approximately 5 ml. of Hanks' minus Ca^{++} and Mg^{++}. The cells were then covered with 5 ml of trypsin or versene solutions. Bottles were gently agitated once or twice during incubation. The cell suspension thus obtained was centrifuged for 3 minutes at 600 rpm, resuspended in a warm hypotonic saline (5% full formula Hanks' with 95% Hanks' minus NaCl) and incubated for 20 to 30 minutes. Centrifugation and decantation followed immediately. A small drop of the cell material was put on a slide, and smear-stained with acetocarmine.

For cells proliferating on cover slips, hypotonic pre-treatment was performed inside the tubes. The cover slips were then removed from tubes, fixed and stained. Both Feulgen and Jacobson's methods of staining, as well as aceto-carmine, proved satisfactory. Temporary slides were rendered permanent by passing them through an ethyl-tertiary butyl alcohol series and mounting in diaphane.

Microscopical Observations. Whenever available, one or more animals of both sexes of a given species were studied. Counts were made of at least 30 good prometaphase or metaphase cells from each species. From 5 to 10 camera lucida drawings were made in each case and averages taken on the measurements of the arm lengths of the highly condensed metaphase chromosomes. Photomicrographs were taken with a Leitz Panphot unit.

RESULTS

Only first passage cultures of trypsinized monkey kidney tissue were examined in these studies. The cell type is predominantly epithelial. In general, mitotic activity begins about one day after inoculation, reaches its peak of about 3% cells in metaphase at 4 to 5 days, and then decreases rapidly. Table 1 shows the observed mitotic index in *Macaca mulatta;* those of other species vary slightly. With appropriate medium renewal and passages, cultures can be maintained for several weeks with retarded mitotic activities. Indeed, Rappaport (1956) has reported recently that in a synthetic medium the same type of cells can be maintained for as long as six weeks.

Within a given species, the chromosome number is remarkably constant in these cultured cells with very occasional polyploid and a few aneuploid cells. Cells with diplochromosomes were observed sporadically. Figures 1 and 2 illustrate such cells from a male *Macaca mulatta* and a female *Erythrocebus patas* showing groups of four homologous chromatids, re-

TABLE 1

MITOTIC INDEX OF KIDNEY EPITHELIAL CELLS (*MACACA MULATTA*) IN CULTURE AT VARIOUS TIMES AFTER INOCULATION.

Days after inoculation	Total cells analysed	Percent of cells at Metaphase
1	519	0.00
2	647	0.46
3	869	0.46
4	567	2.99
5	446	2.24
6	747	0.53

sulting probably from the process of endoreduplication (Levan and Hauschka 1953) during the previous interphase. This process apparently serves as one of the mechanisms of polyploidization (Hsu and Moorhead 1956).

In general, mitoses in all materials appeared to be regular. However, multipolar spindles have been observed in a few instances. Hypotonic treatment swells cells in culture and disturbs the spindles of the dividing cells. There seemed to be no noticeable effects on chromosome morphology using the present tonicity and within the prescribed treatment time. Prolonged treatment over one hour under such conditions usually causes cells to burst.

The chromosome numbers of the nine species examined (including two subspecies in one case) are listed in table 2. Representative photomicrographs of chromosome complements are shown in figures 3–6. The somatic chromosome numbers in *Papio papio* and *Macaca mulatta,* both 42, confirm the report by Darlington and Haque (1955) using testicular materials. The chromosome numbers of the remaining species were determined for the first time in this study, as far as the authors are aware.

Preliminary studies have been made on chromosome morphology in the various species, as a prelude to more detailed quantitative observations to establish precise comparative idiograms. The chromosomes in all these species can be classified into three groups on the basis of chromosome arm indices, as described by Tjio and Levan (1956) for human chromosomes. These three groups are: M chromosomes (median-submedian centromere); S chromosomes (subterminal centromere); and T chromosomes (nearly terminal centromere). The majority of these primate chromosomes are in the M group, only a few falling into the S or T categories. There are no true telocentric chromosomes in these species. The chromosomes at somatic metaphase vary in length from 1 to 9μ, forming a more or less continuous series. The total length of the diploid chromosome complement at metaphase is similar for the various species, despite the range in chromosome numbers, and approximates 100u.

The sex pair (of which there is one in each species examined) was determined only by means of matching all the somatic chromosomes in pairs. The X chromosomes in all these monkey species, like that in man,

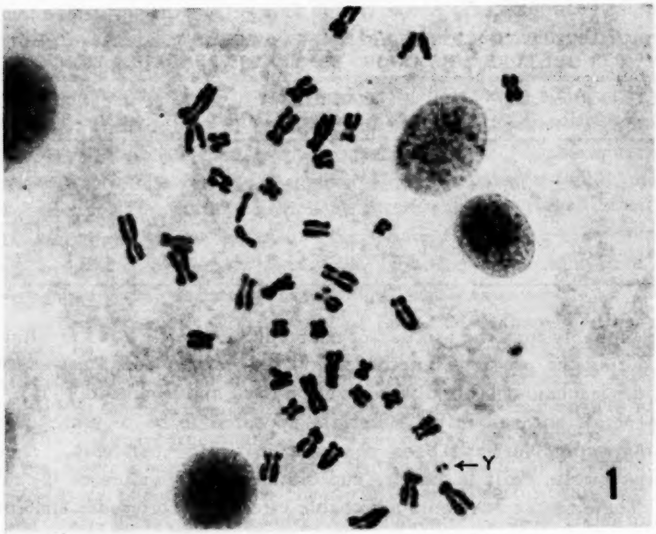

Figure 1. Cell from a male *Macaca mulatta* showing 42 diplochromosomes. Arrow indicates Y chromosome. 1620 X.

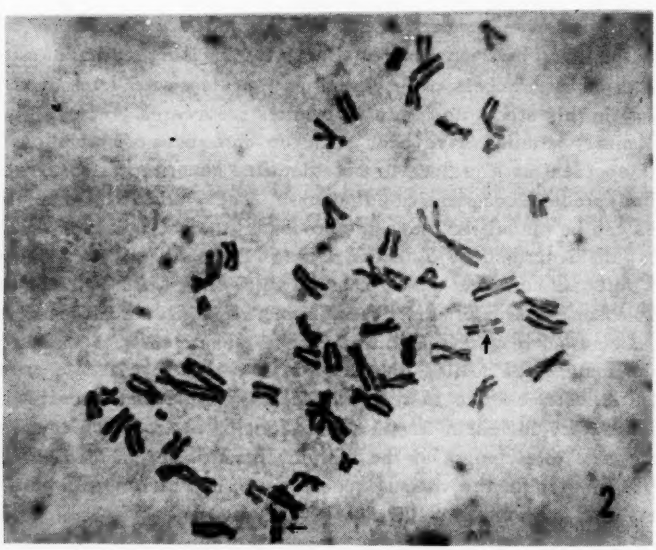

Figure 2. Cell from a female *Erythrocebus patas* showing 54 diplochromosomes. 2040 X.

have submedian centromeres. They are in general much smaller than the human X, being in all cases only slightly larger than the smallest autosome in each genome. The Y chromosomes of all monkeys examined appear to have marked similarities in size and form: they all have nearly median centromeres, measure about 1μ in total length, are the smallest chromosomes of the total complement, and are also smaller than the human Y. This last conclusion agrees with the earlier observation by Darlington and Haque (1955).

Perhaps the best interspecific chromosomal comparison is afforded by the single autosomal pair having a marked secondary constriction. General evidence indicates that this is the single nucleolus-organizing chromosome pair. One such pair is present in each species and the pairs are morphologically alike in the sense that their secondary constrictions are of similar lengths and are located close to the centromere. However, the total length of this particular chromosome pair appears to differ in different species.

DISCUSSION

The present studies indicate that the use of tissue cultures, coupled with the application of suitable modern cytological techniques (such as hypotonic pretreatment), can provide useful information on chromosome number and morphology in various species of Primates. Observations on nine

TABLE 2

SOMATIC CHROMOSOME NUMBERS DETERMINED IN TISSUE CULTURES OF MONKEYS IN THE FAMILY CERCOPITHECIDAE.

Scientific Name†	Common Name	Sex and Number of Individuals Examined		Somatic Chromosome Number
		Male	Female	
Macaca mulatta	Rhesus macaque	3	1	42
Papio papio	Guinea baboon		1	42
Papio doguera	Olive baboon	2		42
Cercocebus torquatus lunulatus	White-crowned mangabey	2		42
Erythrocebus patas	Military red-grass monkey	1	3	54
Cercopithecus aethiops sabaeus	African green monkey	1		60
Cercopithecus aethiops tantalus	African white monkey	2	1	60
Cercopithecus diana roloway	Diana monkey	1	1	60
Cercopithecus mona campbelli	Campbell's monkey		2	66
Cercopithecus nictitans buttikoferi	White nose or spotted nose monkey		3	66

†Species identifications were made by Mr. T. Donald Carter, Department of Mammals, American Museum of Natural History, New York City.

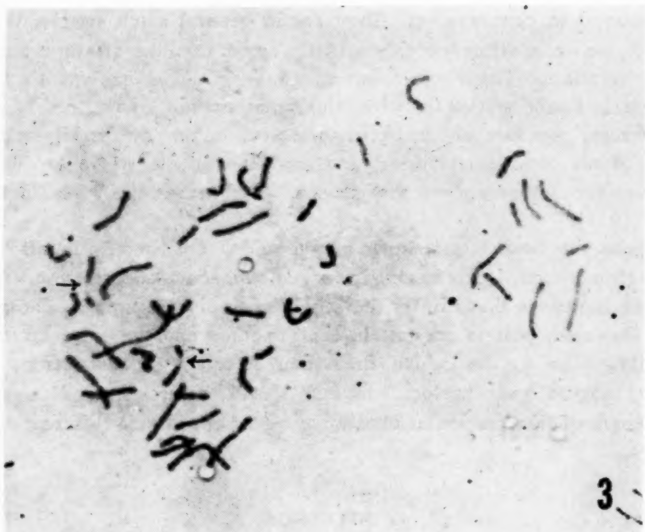

Figure 3. *Macaca mulatta,* female. 2N = 42. Arrows indicate pair of homologous chromosomes with secondary constrictions. 1400 X.

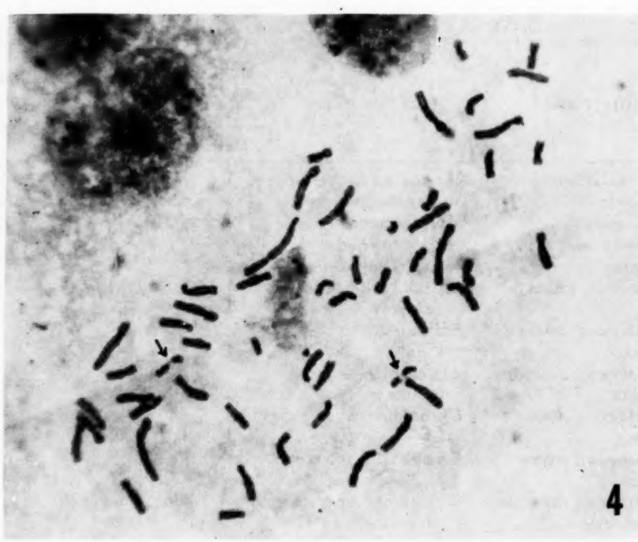

Figure 4. *Erythrocebus patas,* female. 2N = 54. Arrows indicate pair of homologous chromosomes with secondary constrictions. 1675 X.

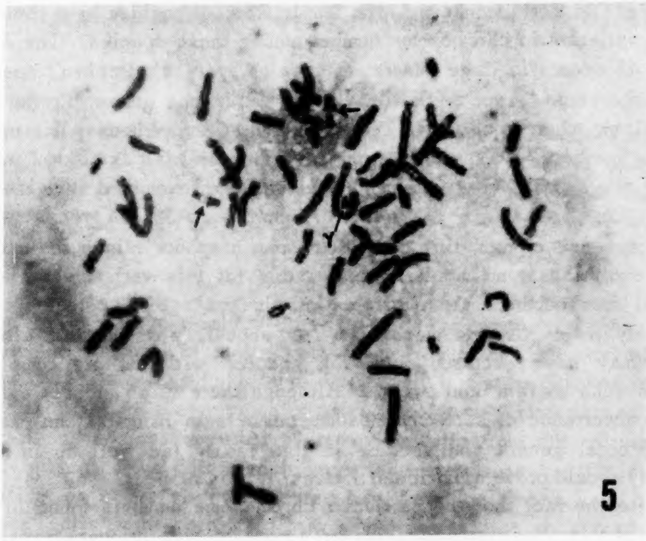

Figure 5. *Cercopithecus aethiops tantalus*, male. 2N = 60. Unlabeled arrows indicate pair of homologous chromosomes with secondary constrictions; labeled arrow indicates Y chromosome. 1890 X.

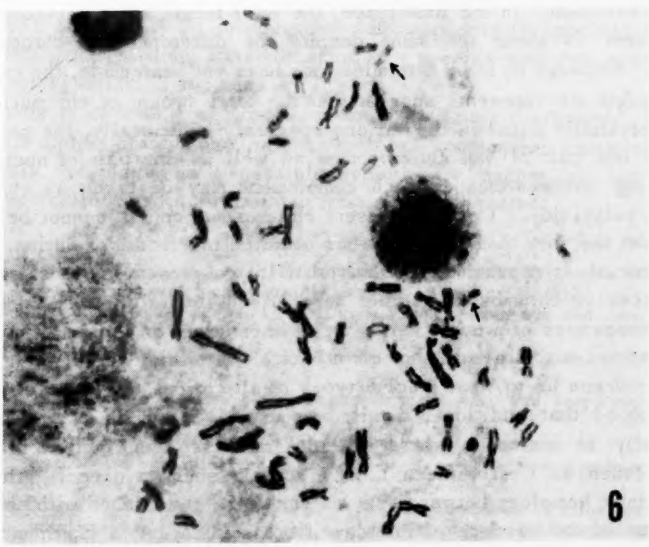

Figure 6. *Cercopithecus nictitans buttikoteri*, female. 2N = 66. Arrows indicate pair of homologous chromosomes with secondary constrictions. 1845 X.

species of Old World monkeys in the family Cercopithecidae have revealed a notable variation in chromosome number among these species. The diploid number 42 occurs in three genera, Macaca (1 species), Papio (2 species), and Cercocebus (1 species); the number 54 in one genus, Erythrocebus (1 species); whereas the fifth genus studied, Cercopithecus, has species with two numbers, 60 (2 species) and 66 (2 species). It is also of interest that the two subspecies of *Cercopithecus aethiops* examined have the same number (60).

The presence of four different chromosome numbers raises the question of the evolutionary mechanisms responsible for this variation. The fact that all four numbers are multiples of six leads to a consideration of polyploidy as a possible mechanism. It appears possible that allopolyploidy may have occurred involving species with haploid complement numbers such as nine and twelve. Although there is no present evidence for the occurrence of such chromosome numbers in Primates, an examination of other genera and species in this family (as well as in related families) should prove a particular interest in this respect.

Despite the fact that the particular chromosome numbers found in these monkey species suggest polyploidy as a possible evolutionary mechanism, there are reasons for believing this interpretation to be improbable. In addition to the general objection that polyploidy should not occur in mammals because of the type of sex-determining mechanism (cf. White, 1954), there is cytological evidence from the present study that this mechanism is probably not responsible for the observed chromosome number variation. In the first place, the total length of each chromosome complement is about the same despite the differences in chromosome number. Although no DNA determinations have yet been made, the chromosome length measurements suggest that the total amount of chromatin may be substantially equal in the various species. Additionally, the presence of only one pair of sex chromosomes as well as one pair of nucleolus-organizing chromosomes in each complement may be taken as evidence against polyploidy. Thus the present chromosome counts cannot be taken to support the view that polyploidy has occurred in Primate evolution.

In general, it appears more likely that in the present group of species differences in chromosome number have arisen from alterations in only a few chromosomes of a basic set, giving either larger or smaller numbers of chromosomes with little loss of chromosomal material. As yet there is no clear evidence as to what precise types of alterations may have occurred. It is hoped that additional detailed comparative studies of karyotypes, especially in instances where different numbers occur within a single genus (such as Cercopithecus), may provide such evidence. Although chromosome homology between species cannot be established with absolute certainty in the absence of interspecific hybridization, a continuation of these comparative cytological studies should make possible at least certain conclusions as to species relationships on criteria other than

morphological, and thus serve to supplement the present taxonomic evidence.

ACKNOWLEDGEMENTS

Grateful acknowledgement is made to Drs. Joseph L. Melnick and G. D. Hsuing of the Yale University School of Medicine for their kindness in furnishing the kidney cells used in these studies. Miss Jean MacCauley has been most helpful as a technical assistant.

SUMMARY

The chromosome numbers and karyological characteristics of nine species of monkeys (including two subspecies of one species) all in the family Cercopithecidae have been determined in tissue cultures of kidney epithelial cells. For cytological studies, two types of materials were used: (1) cell suspensions obtained by removing cells from the culture vessel walls by incubation with trypsin or versene solutions and (2) cells proliferating on cover slips in depression (Leighton) tubes. In both procedures cells were pretreated with hypotonic saline to spread chromosomes and smear-stained with acetocarmine. The chromosome numbers of the materials studied appeared to be essentially constant with very occasional polyploid and aneuploid cells.

Diploid chromosome counts have revealed a notable variation in chromosome number among the species studied. The diploid number 42 occurs in three genera, Macaca (1 species), Papio (2 species), and Cercocebus (1 species); the number 54 in one genus, Erythrocebus (1 species); whereas the fifth genus studied, Cercopithecus, has species with two numbers, 60 (2 species) and 66 (2 species).

Details of comparative chromosome morphology are discussed. The fact that all diploid chromosome numbers in these species are multiples of six suggests polyploidy as a possible evolutionary mechanism, but several lines of evidence against this interpretation are presented.

LITERATURE CITED

Chu, E. H. Y. and N. H. Giles, 1956, Primate chromosome complements determined by tissue culture techniques. (Abstract). Genetics 41: 637.

Darlington, C. D. and A. Haque, 1955, Chromosomes of monkeys and men. Nature 175: 32.

Ford, C. E. and J. L. Hamerton, 1956, The chromosomes of man. Nature 178: 1020-1023.

Hsu, T. C., 1952, Mammalian chromosomes *in vitro*. I. The karyotype of man. Jour. Hered. 43: 167-172.

Hsu, T. C. and C. M. Pomerat, 1953, Mammalian chromosomes *in vitro*. II. A method for spreading the chromosomes of cells in tissue culture. Jour. Hered. 44: 23-29.

Hsu, T. C. and P. S. Moorhead, 1956, Chromosomal anomalies in human neoplasms with special reference to the mechanisms of polyploidization and aneuploidization in the HeLa strain. Ann. N. Y. Acad. Sci. 62: 1083-1094.

Hughes, A., 1952, Some effects of abnormal tonicity on dividing cells in chick tissue cultures. Quart. Jour. Micro. Sci. 93: 207-220.

Levan, A. and T. S. Hauschka, 1953, Endomitotic reproduction mechanisms in ascites tumors of the mouse. Jour. Nat. Cancer Inst. 14: 1-43.

Rappaport, C., 1956, Monolayer cultures of trypsinized monkey kidney cells in synthetic medium. Application to poliovirus synthesis. Proc. Soc. Exper. Biol. Med. 91: 464-470.

Tjio, J. H. and A. Levan, 1956, The chromosome number of man. Hereditas 42: 1-6.

White, M. J. D., 1954, Animal cytology and evolution. Second Ed. Cambridge University Press. London & N. Y.

Youngner, J. S., 1954, Monolayer tissue cultures I. Preparation and standardization of suspensions of trypsin-dispersed monkey kidney cells. Proc. Soc. Exper. Biol. Med. 85: 202-205.

Vol. XCI, No. 860 The American Naturalist September–October, 1957

THE ORIGIN OF THE LARVA AND METAMORPHOSIS IN AMPHIBIA

HENRYK SZARSKI

Department of General Zoology, Copernicus University, Toruń, Poland

INTRODUCTION

The presence of larva and metamorphosis in amphibian development is usually interpreted in a strict Haeckelian sense. According to this point of view the characters present in the amphibian tadpole are a heritage from fish ancestors and the metamorphosis in a short recapitulation of the processes which once drove vertebrates out of the water. This mode of reasoning undoubtedly stimulates research, throwing light on the origin of tetrapods (e.g. Sembrat 1929, 1953, 1954). The correlation between ontogeny and phylogeny is however by no means so simple as Haeckel assumed. This fact has been stressed several times; let us quote only Severtzoff (1927) and de Beer (1940). It is the aim of the present article to bring together and to discuss recent findings relative to the past history of the tadpole and metamorphosis in Amphibia.

The number of publications directly or indirectly referring to these questions is rather extensive. Especially the morphology of the tadpole and amphibian metamorphosis have been studied many times. It is impossible therefore to give here a comprehensive review of the literature. On the other hand, the larva and metamorphosis in Gymnophiona are too little known to be considered in detail.

Among Chordata many forms pass during their development through the stage of a free-living larva. In the majority of Tunicata there is a tailed larva, the morphology of which is considered as the clearest indication of the affinities of the group. A larva is also present in the development of Amphioxus. A very characteristic larval stage is seen in the ammocoetes of lampreys. Among fishes the presence of a larval form is especially characteristic of those groups which have many primitive features, such as Branchiopterygii, Chondrostei, Holostei and Dipnoi. It is therefore very probable that a larval stage was uniformly present in all chordate ancestors. These facts induced Garstang (1928) to assume that the chordates arose from sessile ancestors through neoteny. Garstang's views were further developed by Gregory (1946).

COMPARISON OF FISH AND AMPHIBIAN LARVAE

According to Portmann (1935) the principal common characteristics in the development of some fishes and Amphibia are as follows: first, external impregnation; second, holoblastic segmentation; third, the structure of the egg envelopes; fourth, short duration of embryonic development; fifth, long

lasting larval stage, culminating in metamorphosis; sixth, the morphology of the larva.

The external appearance of a urodele larva does not differ considerably from that of a metamorphosed specimen. The anuran larva has a similar build just after hatching, before the so-called "first metamorphosis", which occurs before the commencement of active feeding. During the first metamorphosis the gills disappear under a skin fold (operculum) and the intestine is coiled in the body cavity. In consequence the animal assumes the characteristic appearance.

All amphibian and fish larvae have a similarly constructed tail fin, consisting of a skin fold. Young larvae do not possess paired appendages. In caudate amphibians the pectoral appendages appear earlier than the pelvic, and the same sequence of events is seen in fishes. In salientians the anterior limbs in the majority of species are hidden in the peribranchial chamber until metamorphosis, but grow at a similar rate as the hind limbs.

The tadpoles of tailless amphibians have at the time of hatching two adhesive organs. These are lost during the first metamorphosis. Freshly hatched caudate tadpoles have in the homologous position two "balancers," which serve as a support for the young animal. The balancers are resorbed when the anterior limbs attain sufficient length to perform the same function (Grodziński, 1929). Baltzer (1952) claimed that the adhesive organs in the Bombina tadpole and the balancers of newts are homologous and cited experimental evidence. Similar structures are present in fish larvae (Kryshanovski, 1949).

Young amphibian larvae are covered with a thin epithelium lacking multicellular glands. A similar epithelium is found on the skin of fish larvae. In the epidermis of Urodela and Gymnophiona characteristic large cells, known as Leydig's cells, are present. During metamorphosis the number of cell layers increases in all Anamnia. The development of multicellular glands often considerably precedes the remaining metamorphic processes, but the disappearance of Leydig's cells ensues at the end of the metamorphosis. The appearance of scales in young fishes is a comparatively late ontogenetic development (Elson, 1939; Everhardt, 1949). The skin capillary net in anuran larvae is situated under the cutis; during metamorphosis it is replaced by another net with smaller meshes, lying between the cutis and the epidermis (Strawiński, 1956).

Skeleton. Both in fish and in amphibian larvae there is an absence of bone tissue, the skeletal parts being formed only of cartilage (Kotthaus, 1933; Sedra, 1950). It is worth special notice that owing to this fact the appearance of bone in some amphibians is very belated. Thus, for example, in *Pelobates fuscus* the bone first appears in the fourth month after hatching, and in *Rana catesbeiana* not until the third year of life (Huxley 1933). The rudiments of the appendicular skeleton and of the pectoral and pelvic girdles display typical tetrapod features from the beginning of their development. Holmgren (1939) tried to show that the developing fore-limb

skeleton of Urodela shows resemblances to the archipterygium, while the early structure of the fore limb of Anura resembles that of the crossopterygium. His reasoning, however, seems unconvincing. Evidence for an opposite point of view is given by Devillers (1954). Similarly, the appendicular amphibian musculature has tetrapod features from the beginning of its appearance.

The Nervous System. In the central nervous system of fish and amphibian larvae so-called giant neurites are present. They spring from Mauthner's cells, situated in the brain and connected centrally by dendrites with the centers of the lateral line system and of the labyrinth. Giant neurites run to the motor centers of the spinal cord. In poorly swimming fishes and in Urodela, Mauthner's cells are present in adults, but disappear during development in those fishes which have succeeded in evolving more efficient swimming reflex patterns, and in Anura (Stefanelli, 1953).

The lateral line system is present in all fish and amphibian larvae. In many fishes the receptors of this sense are situated in canals or pits; in larval forms both of fishes and of amphibians they lie superficially, uncovered. In the majority of amphibians the lateral line receptors disappear during metamorphosis. They are, however, retained in forms which do not leave the water (for example, Xenopus), and in some species undergo only a partial atrophy with subsequent regeneration during the breeding period (for example, Triturus). During metamorphosis of tadpoles the eye-lids appear and the sound-transmitting apparatus undergoes reconstruction (Witschi 1955).

In the majority of Urodela the absence of teeth during the larval period deserves mention. Probably in connection with this the larvae of the genera Ambystoma and Onychodactylus have a "beak", reminiscent of the horny teeth which surrounds the mouth in anuran larvae. The variability of these structures is considerable. They are often used in systematic keys. Perhaps there is some phylogenetic relationship between the cornifications surrounding the mouth apertures of amphibian larvae and of adult lampreys.

The *pharynx* of the anuran tadpole is divided into two chambers by folds of mucous membrane, called the ventral and the dorsal velum. The dorsal chamber is the pharynx sensu stricto, the ventral chamber forms the cavity of the branchial basket, whence the water is passed to the peribranchial cavity. (The translated nomenclature of Schulze [1892] and of Kratochwill [1933], not that of Savage [1952, 1955] is here adopted). On the internal surface of the branchial basket wall is found the filtering apparatus. The gills are connected with the external surface of the wall, projecting into the peribranchial cavity.

The *filtering apparatus* in the tadpole has a highly complicated structure. It can be sketched in the following way. On every gill arch stands a dorsally directed plate. The mucous membrane covering the plates forms a complicated pattern of folds. The folds nearly touch each other, leaving between them only very narrow clefts. Along the bases of the folds run the

collecting channels, opening on the edge of the filter plates to the peribranchial cavity. When the gill slits are closed the water passes between the folds to the collecting channels, leaving the suspended particles on the edges of the folds. Several authors have stated that the filtering apparatus of anuran tadpoles permits them to feed on finely dispersed particles, even on bacteria (Burke, 1933; Kratochwill, 1933; Goldacre, 1949). Recently Dodd (1950) has shown that the tadpoles of *Rana temporaria* and *Bufo bufo* are able to collect dispersed graphite, the grains of which measure from 0.2μ to 2μ in diameter. The transport of particles from the filter plates to the oesophagus is not completely understood. On the dorsal surface of the pharynx rows of ciliated cells are found, along which the mucous cords are propelled to the oesophagus, but there are no cilia on the epithelium covering the folds on the filter plates. Savage (1952) suggests that the mucus containing the particles is moved from the filtering apparatus to the pharynx sensu stricto by water currents formed in the cavity of branchial basket.

Feeding on small particles dispersed in water is a very ancient trait in Chordata. It may therefore be suggested that this mode of life in anuran larvae is inherited from remote ancestors, or at least from their young developmental stages. This view is probably correct. It must, however, be remembered that the filtering apparatus in tadpoles is very different from the structures seen in the pharynx of Amphioxus or of ammocoetes. It would be very interesting to find connecting links; these are probably present in some fish larvae.

Digestive System. A striking fact is the absence of a stomach in anuran tadpoles. Recently this phenomenon was studied by Barrington (1946) and Savage (1952, 1955). In some tadpoles the anterior portion of the intestine forms a "manicotto", a term used by Barrington and Savage. It is a peculiar organ, very different from the vertebrate stomach. During metamorphosis the manicotto disappears and a typical stomach is formed. The functional significance of the absence of the stomach in tadpoles is not clear. I have tried to explain the absence of the stomach in Cyprinidae by the consumption of food rich in buffering salts, especially carbonates. In such circumstances the absence of a stomach causes a considerable economy in anions, particularly of chlorine and phosphorus (Szarski, 1956; Szarski, Delewska, Leja, Olechnowiczowa, Predygier and Siankowa 1956). A similar line of thought will perhaps also explain the absence of a stomach in tadpoles. The deposits of mud on the bottom of fresh-water ponds sometimes contain considerable amounts of carbonates, and the tadpoles, which feed almost continuously take very large quantities of food, which would neutralize the acid in the stomach. The animal is therefore forced either to increase largely its production of hydrochloric acid, or to limit its digestive processes to those which can take place in an alkaline medium The second alternative leads in the long run to a reduction of the stomach.

Another consequence of the ingestion of large quantities of dilute food is the considerable length of the intestine in anuran tadpoles. During metamorphosis the intestine is much shortened.

Circulatory System. The first rudiments of blood vessels appear on the yolk-sac both in amphibians (Grodziński 1924) and in fishes (Kryżanowski 1933). During the first developmental stages in both groups the yolk sac surface plays a dominant role in the respiratory exchange (Olko, 1955; Strawiński, 1956). The blood vessels of tadpoles are arranged in a pattern essentially similar to that of fishes. This fact is often quoted as a proof that the anatomy of the tadpole corresponds to that of its fish ancestors. It must be stressed that in tadpoles the postcardinals convey the blood to the portal circulation of the pronephros. A similar construction is found in larval, but not in adult, fishes.

The circulation in the lungs of tadpoles does not differ to any great degree from that in the lungs of Dipnoi. An interesting fact is the very early appearance of rudiments of lymphatic sacs in the development of Anura. These structures, characteristic of the whole order, are formed long before metamorphosis (Hoyer 1934).

A very characteristic feature of fish and amphibian larvae is the presence of external gills. These are found in the larvae of Elasmobranchii, Chondrostei, Holostei, Dipnoi and Teleostei, as also in all amphibians. In Urodela they persist until metamorphosis. In Anura they are reduced in the course of the first metamorphosis. In neotenous Urodela the external gills are retained for the whole life (for example, Typhlomolge, Proteus). The internal gills of Anura according to Schmalhausen (1954), are homologous with external gills. Their arrangement is very variable in different forms; it is however always quite different from the gills of fishes. Thus, in tadpoles of the edible frog, the gills are branched in tree-like fashion (Strawiński, 1956). The tadpoles of *Xenopus laevis, Glyptoglossus molossus* and *Calluella guttalata* have no gills (Savage, 1955). The quantitative investigations of Strawiński (1956) demonstrate that even in the edible frog, where the gills are fully developed, the part they play in respiratory exchange is always rather small. The principal part is played by the body surface, the second place being taken by the early developing lungs. At the time of the greatest relative dimensions of the gill vessels, they form only 27.9 percent of the total length of the respiratory capillaries. The tadpoles at that time have still no hind legs. Immediately before metamorphosis this value drops to 13 percent.

Excretion. The pronephros is the larval kidney of all fishes and amphibians. In fishes it disappears very early, its function being taken over by the mesonephros although there are species belonging to the genera Cyclothone, Hemirhampus and Lepadogaster in which the pronephros is retained in sexually mature specimens. These species are usually considered neotenic. The amphibian pronephros contains nine to eleven nephrons in Gymnophiona, two to four in Urodela, three in the genus Rana, etc. The mesonephros appears in tadpoles before metamorphosis, the pronephros disappears at the beginning of this process. In Gymnophiona the reduction of the pronephros takes place directly after hatching, during the first metamorphosis (Gérard, 1954).

Most fishes dispose of their nitrogen wastes in the form of ammonia, while adult amphibia produce urea (Delaunay, 1931). Tadpoles excrete ammonia, but in the course of metamorphosis they acquire the ability to synthesise urea. Underhay and Baldwin (1955) stated that in *Xenopus* laevis, an amphibian spending whole of its life in water the production of urea appears at the beginning of metamorphosis. It is subsequently lost in the course of the process. The adult animal excretes ammonia like a tadpole.

Genital System. The only organ not affected by metamorphosis in amphibians are the gonads. The absence of correlations between the degree of gonad development and the state of the other organs allows the presence of neoteny, which is of rather common occurence in Amphibia. Jurand (1955) describes larvae of *X. laevis* which had congenital absence of the thyroid gland. They lived for seventeen months, reaching 125 mm in length. Their gonads were well differentiated, while all other organs retained larval characters.

SUMMARY OF COMPARISONS OF AMPHIBIA AND FISH

The principal features of a typical amphibian tadpole have been compared above with many structures of a fish larva. It must, however, be remembered that many amphibian larvae differ significantly from the build considered typical, and that many species have no larval stage. These facts were reviewed by Barbour (1923), Noble (1931) and Angel (1947). Let us repeat some points.

Among Gymnophiona the genera Hypogeophis, Dermophis, Siphonops and Typhlonectes do not possess a larval stage. In Hypogeophis the whole development is passed in the egg envelopes; in Typhlonectes, Dermophis and Siphonops the female gives birth to fully-developed offspring. Lungless salamanders belonging to the family Plethodontidae lay eggs on land. From the egg there hatches a metamorphosed animal. In the genera Hydromantes, Oedipus, and in the Alpine salamander (*S. atra*), the whole development is passed in the oviducts of the female. The life history of *Amphiuma tridactylum* is particularly striking. This animal lives in water, the sexually mature specimens showing some larval traits, for example, open gill slits and absence of eye-lids; they do not, however, possess gills and their skin is metamorphosed. These amphibians leave the water only during the breeding period, when they lay eggs on land. Young salamanders enter the water directly after hatching (Baker, 1937).

Many tailless batrachians have no larval stage. In Nectophrynoides, a bufonid living in Western Africa, the whole development is passed in the spacious oviducts. In many forms, eggs laid in humid environments produce animals similar to the parent. The development of several species of the genus Eleutherodactylus was described by Gitlin (1944), Lynn (1948) and Lynn and Lutz (1946). Even in the genus Rana, *R. opisthodon,* a species inhabiting the Solomon Islands, has no larval stage. The female lays eggs

in crevices between stones and from the eggs young froglets emerge. This fact is explained by the scarcity of stagnant water in the region (Van Kampen's theory).

SIGNIFICANCE OF DIRECT DEVELOPMENT

It is usually assumed that direct development in Amphibia is a secondary adaptation. An opposite view is put forward by Stephenson (1951 a,b) in his publications dealing with the development of the primitive salientians Leiopelma and Ascaphus. The eggs of these animals are large and rich in yolk. The egg of Ascaphus measures 4-5 mm, and that of Leiopelma 5 mm in diameter. Leiopelma lays eggs on land. During the development four gill slits are opened; there are, however, no gills and no operculum. Only a collar on the neck is formed, somewhat similar to a structure found in Urodela. Horny larval teeth are absent. A young Leiopelma, leaving the egg envelopes, has four well-developed legs and a powerful tail, which aids in breaking the egg capsule. The intestine is never coiled, and yolk is present in it even in the fourth week after hatching. At this moment the young Leiopelma has four arterial arches and a well developed pronephros, which disappears after some weeks. The resorption of the tail takes about a month. The lungs are rudimentary and begin their function some weeks after hatching. The only respiratory surface of a young specimen is formed by the skin.

The female of Ascaphus lays her eggs in water. The tadpole hatches a whole month after the deposition of the eggs, and has a length of 13.5 mm. The Ascaphus tadpole has an operculum covering the gills and fore-limbs. During metamorphosis this structure is not perforated, as in the majority of salientians, but the legs emerge through a very large median spiracle.

METAMORPHOSIS IN FISHES AND AMPHIBIANS

The processes called metamorphosis are in reality very different in different forms. Spurway (1953) writes: "it cannot be overemphasized that the use of a singular noun 'metamorphosis' for these processes is unfortunate (Noble 1931)."

In spite of many attempts it has been impossible to show that during metamorphosis of Tunicata, Acrania or Cyclostomata, factors similar to those responsible for amphibian metamorphosis are acting. There is one interesting experiment of Sembrat (1953), who succeeded in obtaining metamorphosis in axolotls by the implantation of lancelet endostyles.

An interesting account of the development of a sturgeon, *Acipenser stellatus* is given by Olifan (1945), who stresses its similarity to amphibian development. A "prelarval stage" lasts for four days after hatching. The larval stage is finished on the 10th day, and metamorphosis follows. On the 26th day metamorphosis is completed. According to Vasnietsov (1948), alternating periods of growth and morphogenesis can be observed in the development of all species of fishes. The periodicity in development

corresponds to changes in diet, which depend on the size of the larva. The animals forming the food of a young fish have several morphological and ecological traits, to which not only the alimentary canal, but the whole fish organism must be adapted. Let us discuss the development of the bream (*Abramis brama*) as an example. At the time of hatching the animal is about 5 mm long, the mouth is not perforated, the fold of skin forming the unpaired fins is not differentiated, the pectoral fins are rudimentary. This is the prelarval stage, or the stage of free embryo. The larval stage is more than 6 mm long, and has a swimming bladder filled with gas, so its swimming ability is greater. The unpaired fins begin to differentiate, and the pectoral fins enlarge. The larva feeds on immobile or slightly mobile organisms. When the fish is 9 mm long, fin-rays appear in the tail fin, and a little later also in the dorsal and anal fins. The larva then feeds on actively swimming organisms. The final transformation is reached only when the animal begins to take the bottom fauna.

Under the influence of Vasnietsov, similar alternating periods of growth and morphogenesis were described in the development of several fishes. Lange (1950) described 10 periods in the development of the loach, (*Nemachilus barbatulus*). Vernidub and Gusieva (1950) divided the development of the roach (*Rutilus rutilus*) into eight periods. They showed that during the periods of growth the daily gain in length attains 4.92–5.51 percent while in the periods of metamorphosis it drops to 0.3 percent. Ieremieieva (1950) describes similar facts in the development of other fishes.

Hormones in Metamorphosis. The possible dependence of fish metamorphosis on thyroid hormone has been investigated several times. Vrtel (1931) demonstrated that in selachian embryos, having large external gills, the thyroid gland produces a secretion which enters the blood stream. This process is, however, greatly intensified just before hatching, after the resorption of the yolk sac. Many glandular alveoli then collapse and are resorbed. The multiple morphogenetic processes going on at this time in selachian embryos can be compared to amphibian metamorphosis.

Olifan (1945) writes that in *Acipenser stellatus* the thyroid gland shows a distinctly higher activity during metamorphosis. Gerbilski and Sachs (1947) obtained an acceleration of metamorphosis in the same species by feeding larvae with thyroid. Irikhimovitch (1948a,b, 1950) disputes their findings. According to Irikhimovitch the thyroid gland is formed very early in *A. stellatus* and early begins the synthesis of the hormone, but its secretion into the blood-stream begins late, only after the pituitary comes into action, which ensues after the completion of metamorphosis. According to this worker the dependence of metamorphosis on the thyroid gland is first seen in Amphibians.

Several facts, however, argue against the conclusions of Irikhimovitch. Sembrat (1954) obtained a chemical thyreotomy by feeding young sea-trout with methylothiouracil. The experimental animals were smaller than the controls, their skin was thinner and more darkly pigmented. A striking

fact was the complete absence of scales in fishes seven months old. In one-year old animals, scales were found only on the body sides, but were absent from the back and belly. This is evidence that there are morphogenetic processes in fish development which are dependent on thyroid gland function.

Salmon and sea-trout pass through a second metamorphosis between the parr and the smolt stages. A number of investigators have shown the correlation between smoltification and thyroid gland function. Fontaine and Olivereau (1947) and Robertson (1948) described the activation of the gland occurring in this period. La Roche (1950) obtained artificial smoltification by thyroid administration. The results were confirmed by La Roche, Leblond and Préfontaine (1950), and by other workers. A review of the findings is given by Olivereau (1954).

The metamorphosis of the eel was very thoroughly studied by von Hagen (1936). The first metamorphic processes (loss of larval teeth, displacement of anal aperture) take place during a period of slow growth of the thyroid gland. A rapid emptying of the gland from the accumulated secretion ensues only at the end of metamorphosis, at the time of entry into fresh water. Von Hagen considers that the activation of the glands of internal secretion is a result of a change in the environment and is not connected with morphogenetic processes. Vilter, however, stated (1945 a,b) that the extirpation of the pituitary arrests the metamorphosis of the skin in eel larvae, and that the larvae forced to remain in salt water have no metamorphic changes in the alimentary canal.

Buchmann (1940) showed that during the metamorphosis of herring the thyroid gland enlarges by building new alveoli. Murt and Sklower (1928) concluded that the metamorphosis of the plaice, *Pleuronectes platessa,* is dependent on the thyroid. They had, however, a very limited material at their disposal and their findings require confirmation.

Sachs and Zamkova (1947) failed to obtain any changes in young larvae of *Misgurnus fossilis* up to the 13th day after impregnation under the influence of thyroxin. Older larvae react to thyroxin by an earlier resorption of the external gills and yolk sac, by a greater mobility of the intestine, and by an earlier production of pigment in the skin and eyes. Thiourea did not influence the development of Misgurnus. Sachs and Zamkova therefore, reach the conclusion that although the early developmental stages react to thyroxin, its presence is not obligatory in the course of morphogenesis.

Goldsmith, Nigrelli, Gordon, Charipper and Gordon (1944) obtained with thiourea an arrest of growth and of sexual differentiation in crosses of *Xiphophorus helleri* with *Platypoecilus maculatus.* This fact is emphasised, as in many amphibians the development and activity of the gonads is not related to thyroid function.

Klumpp and Eggert (1935) described the development of *Ichthyophis glutinosus,* which belongs to Gymnophiona and passes through a larval form in its development. This species passes through two metamorphoses,

like many Anura. The first takes place shortly after hatching. The external gills and the pronephros are then reduced. The second metamorphosis is a very long process. It begins when the animal is 9.5 to 10 cm long, and is completed when the length of 16 cm is attained. Unfortunately no exact duration in time is given. The principal processes, taking place in the course of the second metamorphosis are the closing of the gill slits, the development of multicellular glands in the skin, and the thickening of the epidermis. At the beginning of the second metamorphosis the thyroid gland shows a lowered activity. The gland becomes more active in animals 12 to 13 cm long, and reaches the peak of secretory activity in animals about 14 cm long. After the completion of metamorphosis the thyroid returns to a resting condition.

The metamorphosis of Urodela is similarly prolonged. It is difficult to fix the exact limits of duration as there is a great individual variability in every species. Thus, in the European salamander some specimens metamorphose in 8 to 12 weeks, while others stop in some intermediate stage and remain in it for several months. Goux (1945) emphasizes that the definitive coloring of the skin appears much earlier than the metamorphic changes in the internal organs. The multicellular skin glands, the appearance of which is sometimes regarded as the first sign of incipient metamorphosis, are visible usually considerably earlier than the definite pigmentation.

In some Urodela the metamorphosis is never completed. It was found in such cases that there is no lack of thyroid hormone, but the tissues do not react to its presence. For example, in the olm, *Proteus anguineus* and in the mud-puppy, *Necturus maculatus,* treatment with thyroxin will not induce metamorphosis of the skin (Hogben, 1929). In the genera Siren, Cryptobranchus, Megalobatrachus and Amphiuma the skin metamorphoses under the influence of the thyroid hormone, but some larval features remain in these animals for their whole life (Noble, 1931). On the other hand the hyoid bone of Necturus and of Amphiuma never has a larval character, but is from the first stages of development a "metamorphosed" organ.

Aneides aeneus is a salamander laying its eggs on land. From the eggs hatch young specimens having a metamorphosed appearance. Dent (1954) found that the thyroid gland in this species begins to secrete immediately before hatching. A complete destruction of the thyroid, obtained by immersing the embryos in a solution of radioactive iodine, does not prevent a normal morphogenesis. Hence it may be stated that in Urodela there is no one morphogenetic process which would be dependent on thyroid function in all species.

Metamorphic processes progress at a much greater rate in Anura; they are more synchronised and more dependent on thyroid hormone. However, it must again be remembered that in many Anura there is no tadpole stage. Lynn (1938) and Lynn and Peadon (1955) investigated the thyroid function in the direct development of *Eleutherodactylus ricordii* and *E. martinicen-*

sis. They found that the activity of this gland is indispensable for the resorption of the tail, differentiation of the digits etc., but the development of jaws and many other features are independent of thyroid stimulation, notwithstanding that they are dependent on it in the remaining genera of Anura. The common occurence of direct development in Anura shows that the dependence of metamorphic processes on thyroid even in this group is rather variable.

THYROID FUNCTION IN FISHES

Hirschler (1924) wrote that amphibian metamorphosis shows that in the phylogenetic development of tetrapods the thyroid gland played a similar role to that which it plays in recent forms during ontogenesis. Thyroid hormone was once, according to him, a principal stimulus to a phylogenetic process, as now it constitutes a stimulus to ontogenetic development. Very similar views were advocated by Harms (1929, 1934, 1935), who studied thyroid function in some tropical fishes. It is therefore appropriate to quote here some results relative to thyroid function in fishes. A good survey of this extensive field of research is given by Olivereau (1954).

Important results concerning this question were obtained by Sembrat (1929). He has shown that the thyroid gland of carp and of dogfish implanted in tadpoles of common frog (*Rana temporaria*) provokes metamorphosis. Koch and Heuts (1943) and Heuts (1943) found that in *Gasterosteus aculeatus* and in *G. pungitius* thyroid treatment lessens the capacity to withstand a greater salinity in the surrounding water. This capacity is lowered also during the breeding activities, according to these authors as a result of a higher level of thyroid hormone concentration in the blood. Olivereau (1950) stated that *Cyprinus carpio* and *Amiurus nebulosus* transferred from fresh to brackish water (concentration of 0.85 percent) show a lowered thyroid function. After some weeks of stay in this salinity an adaptation is observed, consisting in the returning of the gland to its normal appearance. In the marine fishes *Maraena helena* and *Labrus bergylta*, Olivereau (1948) obtained a pronounced rise in the thyroid activity by the action of brackish water. Hoar (1952), comparing the state of the thyroid in migratory fishes during the life cycle, found that the gland secretes at a greater rate during the stay in fresh water. Hoar suggests that death in the breeding grounds which occurs regularly in some migratory species may be a result of the depletion of iodine in the thyroid gland.

The role played by the thyroid in the migrations of the lower vertebrates is great. Fontaine, Gorbman, Leloup and Olivereau (1952) demonstrated with the use of radioactive iodine that the thyroid of lampreys secretes actively at the commencement of reproductive migration. Similar facts were found also in salmon by Fontaine, Lachiver, Leloup and Olivereau (1948). Sturgeons have enlarged thyroids and a higher metabolic rate during migration. In captured females the eggs do not mature unless the animal is given thyroxin (Biekhtina, 1947; Skadovski, 1949).

In the viviparous Torpedo, Olivereau (1949 a) found a rise in the activity of the thyroid in gravid females. In oviparous Scyllium the peak of thyroid activity is reached when the eggs pass through the segment of oviduct producing the egg envelopes (Olivereau, 1949 b). Scott (1953) has shown that the thyroid hormone is necessary for sexual maturity of the teleostean *Brachydanio rerio.* Hopper (1952) succeeded in accelerating the sexual development of the viviparous fish *Lebistes reticulatus* by the administration of thyroid, and Stolk (1951) ascertained that in this species the activity of thyroid is high during the gestation period, and declines at its end, when the glands of the embryos begin to function. According to Leloup (1947, 1948, 1949) there is a close connection between the function of the thyroid in fishes and the level of copper in the blood.

All these facts prove that in fishes several important physiological processes are dependent on the thyroid gland. On the other hand, it has been several times demonstrated that it is very difficult if not impossible to obtain in these animals any symptoms caused by thyroid overdosage (Fleischmann 1947, Goldsmith 1949). It is therefore necessary to repeat the experiments of Harms (1929, 1935) who claimed that hypertrophy of the thyroid gland is provoked by thyroid administration in the genera Periophthalmus, Boleophthalmus and Blennius. Experimental animals have shown, according to this author, several morphological and physiological changes interpreted as an adaptation to life out of water.

GENERAL CONCLUSIONS

From the facts summarized above several conclusions may be drawn. In the first place it is obvious that the presence of a larva in development is a very ancient trait of Ichthyopsida. The larvae of these animals have many common features, such as the shape of the tail fin, the presence of external gills, of the pronephros, the absence of bone tissue, etc.

Development through a larval stage must be concluded by metamorphosis. In agreement with Vasnietzov (1948) and with Stephenson (1951 b) we may assert that the greater are the differences in the environment, in the mode of life, and in the food between the larva and the imaginal form, the more striking is the metamorphosis.

The progress in the mode of development of Amphibia must have been considerable, as natural selection acts on these animals principally during embryonic and larval development. Savage (1952) concludes from his own observations and from the literature that the number of frogs and toads in the British Isles is determined by the quantity of metamorphosing specimens. A large quantity of available food and scarcity of predators would allow a much greater population density. Progress in the mode of development is based on modifications, therefore many traits in tadpoles must be comparatively recent acquirements.

Balaban (1953) tried to classify the features of ammocoetes in three groups, namely, ancestral features, caenogenetic adaptations and retarda-

tions in development. A somewhat similar classification can be adapted to larval traits and the following groups can be discerned.

1. Larval caenogenetic peculiarities. Here we shall collect such features as are common in the early larvae of Ichthyopsida. They can be further divided into:

a. disappearing before the final metamorphosis (suckers in Anura, balancers in Urodela, external gills in Anura and Gymnophiona, pronephros in Gymnophiona).

b. disappearing in the course of final metamorphosis (Leydig cells in epidermis of Urodela, external gills in Urodela, horny teeth, filtering apparatus, spirally winded intestine in Anura).

2. Retardations in development, or prolongations in the persistance of larval traits. Comparison of fish and amphibian larvae shows that these features disappear early during the metamorphosis of fishes. In some amphibians, however, they may last for yeats or, in neotenous species, even persist in sexually mature individuals. These are: few cell layers in the epidermis, cartilaginous skeleton, shape of the tail fin, external gills.

3. Amphibian characteristics which are present in tadpoles. These are always absent from fish development. We may enumerate the structure of the skeleton, muscles, nerves and vessels of paired limbs in Urodela and Anura, and the rudiments of lymphatic sacs in Anura.

4. Amphibian characteristics which appear late in development true anabolies in the nomenclature of Severtzoff (1927). These are never seen in fishes. These are: multicellular skin glands, reconstruction of arterial arches, presence of eye-lids and tympanic membranes, loss of gills, closing of gill slits, loss of the tail in Anura.

5. Characters in Amphibia independent of thyroid function: developmental state of gonads.

The Crossopterygian ancestors of Amphibia, like the majority of fishes, must have passed a larval stage in ontogeny. Their larva may have been very similar to a young urodelan larva or to the larva of recent Dipnoi. The common occurence of larvae in Ichthyopsida and the large quantity of similar traits in all these forms are in favor of these assumptions.

As in recent fishes, the larval stages lasted a short time and the animal was of small size at the beginning of metamorphosis. The processes leading to the loss of larval characters and to the development of imaginal features progressed slowly and independently of each other, as in the skin, in the skeleton, in the blood vessels. They were not so well correlated as in recent Anura, and probably were even less interdependent than in recent Urodela and Gymnophiona. In adults as in recent fishes, the thyroid gland played many important functions. It must have been connected with the regulation of growth processes, with the maintenance of ion balance and internal osmotic pressure, and especially with the proliferation of the epidermis. The great differences in the degree of dependence of various morphogenetic processes on the thyroid, which we observe in recent Am-

phibia, prove that this dependence is not in every instance an ancient phenomenon.

In the early Palaeozoic amphibians natural selection acting very intensively on larvae and on young specimens, markedly favored individuals showing the following traits.

a. Prolonged duration of the larval stage. Owing to this feature the animals were of larger size at the time of transition to land life. This was of great biological value, as large dimensions give a better volume- to-surface ratio, and diminish the danger of drying up.

b. Appearance of caenogenetic larval adaptations such as filtering apparatus in Anura. The importance of these features consisted mainly in allowing a quicker growth rate.

c. Acceleration and synchronization of morphogenetic processes going on at the time of metamorphosis. This shortens the stage during which the individual is adapted neither to land nor to water surroundings, and is therefore most exposed to dangers.

Some morphogenetic processes were probably dependent on the thyroid gland even in Crossopterygians, for example, proliferation of the epidermal cells. As the larval specializations progressed and the number of larval adaptations grew, so did the number of metamorphic processes dependent on thyroid activity. As a striking example of a process which must have appeared comparatively recently, we can point to the resorption of the tail in Anura.

The rise in the importance of the thyroid gland in metamorphosis brought about a tendency to delay the onset of its activity. This tendency produced gigantic tadpoles, many times larger than the largest fish larvae, such as in *Pelobates fuscus, Pseudis paradoxa* and *Rana catesbeiana,* which metamorphoses in the third year of life (Huxley, 1923). The same tendency acted in the production of neoteny.

Paul Weiss (1939, p. 442) wrote that development can be compared to a photographic process. But "the developer does not create the picture; it simply converts a latent picture into a visible one". . . . . . "applied to the organism this means that hormones. . . . . . expose and modify existing differences, but they cannot create new ones. Thus they cannot be primary factors of organization." The comparison of Weiss is useful in the interpretation of amphibian metamorphosis. The role of thyroid in this process consists in the synchronization and coordination of various morphogenetic processes, and it may be large or small according to the amphibian species observed.

The assumption that the origin of Amphibia was evoked by an increase of the internal secretion of the thyroid in a group of fishes must be regarded as completely unjustified. Similarly it is improbable that the early amphibians had a direct development, and that the tadpole and metamorphosis are recent acquirements, as is suggested by Stephenson, (1951 a,b). Striking similarities in the structure of the larvae of Anura, Urodela, and of various

fishes are probably based in the majority of cases on true homologies. Stephenson is, however, right in asserting that many characters peculiar to anuran larvae, and absent in the larvae of fishes and Urodela, are recent adaptations. Finally we may say that the suggestion of Hamlett (1933) that the common ancestors of fishes and amphibians had the appearance of a tadpole is based on false premises.

SUMMARY

All larvae of Ichthyopsida show a great similarity in many important traits. The amphibian larvae, however, have a considerable number of characteristics, which can be considered as comparatively new caenogenetic adaptations. They are most numerous in larvae of Anura.

In many fishes having a larval stage in development there is also a metamorphosis more or less similar to the amphibian one. The influence of the thyroid on fish metamorphosis is very variable and never so pronounced as in Amphibia. The dependence of metamorphosis on the thyroid secretion is greatest in Anura. It is much smaller in Urodela and Gymnophiona. In these two groups metamorphosis is a slow and poorly coordinated set of processes.

The following conclusions can be drawn from these facts. The Crossopterygian ancestors of Amphibia must have had a larval stage in their development, very similar to the larvae of recent Dipnoi and Urodela. A great evolutionary gain was achieved by Amphibia through the prolongation of the duration of the larval stage, by evolving larval adaptations and by a shortening and synchronization of metamorphosis. Probably some of the morphogenetic processes, forming part of metamorphosis of recent amphibian larvae were dependent on the thyroid hormone secretion in Crossopterygians, just as they depend on it in recent fishes. Other processes developed the dependence on the thyroid comparatively recently. A striking example is the resorption of the tail in Anura.

The thyroid gland could not have been a factor in the origin of Tetrapoda from their fresh-water ancestors.

LITERATURE CITED

Angel, F., 1947, Vie et moeurs des amphibiens. Payot, Paris.

Baker, L. C., 1937, Mating habits and life history of *Amphiuma tridactylum* Cuvier and effects of pituitary injections. J. Tenn. Acad. Sci. 12: 206–218.

Baltzer, F., 1952, Experimentelle Beiträge zur Frage der Homologie. Xenoplastische Transplantationen bei Amphibien. Experientia 8: 285–297.

Balaban, P. P., 1953, K morphologitsheski kharateristike lishinki minogi. Zool. Zhurn. 32: 638–641.

Barbour, T., 1923, Reptiles and amphibians. Their habits and adaptations. London.

Barrington, E. J. W., 1946, The delayed development of the stomach in the frog, *R. temporaria* and the toad, *B. bufo*. Proc. Zool. Soc. London. 116: 1–21.

Biekhtina, W. G., 1947, Gistologitsheskoie isledovanie shtshitovidnoi shelezy osetrovykh ryb v period nerestnoi migracii, neresta i pokata. Tr. Labor. Osnov Rybovod. 1 (Quoted after Skadowski 1949).

Buchmann, H., 1940, Hypophyse und Thyroidea im Individualzyclus des Herrings. Zool. Jahrb. Jena. 66: 193-261.

Burke, V., 1933, Bacteria as food for vertebrates. Science 78: 194.

De Beer, G. R. 1940, Embryos and ancestors. Oxford.

Dent, J. N., 1954, Observations on iodine metabolism in embryos of the terrestrial salamander *Aneides aeneus*. Anat. Rec. 118: 294.

Delaunay, 1931, Biol. Rev. 6: 265 (Quoted after Underhay and Baldwin 1955).

Devillers, Ch., 1954, Origine et évolution des nageoires et des membres. P. P. Grassé, Traité de Zool. 12: 710-790.

Dodd, J. M., 1950, Ciliary feeding mechanism in anuran larvae. Nature, 165: 283.

Elson, P. F., 1939, Order of appearance of scales in the speckled trout. J. Fish. Res. Bd. Canada. 4: 302-308.

Everhardt, W. H., 1949, Body length of the smallmouth bass at the scale formation. Copeia 1949: 110-115.

Fleischmann, W., 1947, Comparative physiology of the thyroid hormone. Quart. Rev. Biol. 22: 119-140.

Fontaine, M., Lachiver, F., Leloup, J. et Olivereau, M., 1948, La fonction thyreoïdienne du saumon (*Salmo salar* L.) au cours de sa migration reproductrice. J. Physiol. 40: 182-184.

Fontaine, M., Gorbman, A., Leloup, J. et Olivereau, M., 1952, La glande thyroïde de la lamproie marine (*Petromyzon marinus marinus* L.) lors de sa montée reproductrice. Ann. d'Endocrin. 13: 55-57.

Fontaine, M., et Olivereau, M., 1947, La glande thyroïde du saumon atlantique (*Salmo salar* L.) femelle au cours de sa vie en eau douce. C. R. Ac. Sci. Paris. 224: 1660-1662.

Garstang, W., 1928, The morphology of the Tunicata and its bearing on the phylogeny of the Chordata. Quart. J. Micr. Sci. 72: 51 (Quoted after Hardy 1954).

Gérard, P., 1954, Organes uro-génitaux. P. P. Grassé. Traité de Zool. 12: 974-1021.

Gerbilski, N. L., and Sachs, M. G., 1947, Vliianie tiroksina na postembrionalnoie razvitie sievrugi (Acipenser stellatus). Dokl. Ak. N. USSR. 55: 667-670.

Gitlin, D., 1944, The development of *Eleutherodactylus portoricensis*. Copeia 1944: 91 (Quoted after Stephenson 1951 a).

Goldacre, E. J., 1949, Surface films on natural bodies of water. J. Anim. Ecol. 18: 36-39.

Goldsmith, E. D., 1949, Phylogeny of the thyroid, descriptive and experimental. Ann. N. Y. Acad. Sci. 50: 283-313.

Goldsmith, E. D., Nigrelli, R. F., Gordon, A. S., Charipper, H. A., and Gordon, M., 1944, Effect of thiourea upon fish development. Endocrin. 35: 132-134.

Goux, L., 1945, Note sur un cas de dissociation du complexe morphogénétique de la métamorphose chez la salamandre terrestre. C. R. Soc. Biol. 1939: 819-820.

Gregory, W. K., 1946, The roles of motile larvae and fixed adults in the origin of the vertebrates. Quart. Rev. Biol. 21: 348-364.

Grodziński, Z., 1924, Über die Entwicklung der Gefässe des Dotterdarmes bei Urodelen. Bull. Acad. Polon. Sci. Sér. B. 1924: 57-67.

1929, Das Rusconische Organ bei *Triton cristatus*. Bull. Acad. Polon. Sci. Sér. B. 1928: 455-460.

Hagen, F. v., 1936, Die wichtigsten Endokrinen des Flussaals. Zool. Jahrb. Abt. Anat. 61: 477-538.

Hamlett, G. W. D., 1933, Amer. Natur. 67 (Quoted after Portmann 1935).

Hardy, A. C., 1954, Escape from specialization. In "Evolution as a process" ed. J. Huxley. London 1954: 122-142.

Harms, J. W., 1929, Die Realisation von Genen und die consecutive Adaption. I. Phasen in der Differenzierungen der Anlagekomplexe und die Frage der Landtierwerdung. Z. wiss. Zool. 133: 211-397.

1934, Wandlungen des Artgefüges unter natürlichen und künstlichen Umweltbedingungen. Akad. Verlagsb. Tübingen.

1935, Die Realisation von Genen und die consecutive Adaption. 4 Mitteilung. Experimentell hervorgerufene Medienwechsel: Wasser zu Feuchtluft bzw. zu Trockenluft bei Gobiiformes (Gobius, Boelophthalmus und Periophthalmus). Z. wiss. Zool. 146: 417–462.

Heuts, M. J., 1943, La régulation osmotique chez l'epinochette, *Pygosteus pungitius* L. Ann. Soc. R. Zool. Belg. 74: 99–105.

Hirschler, J., 1924, Gruczoły wewnętrznego wydzielania, a rozwój rodowy zwierząt kręgowych. Przyr. i Techn. (Quoted after Sembrat 1929).

Hoar, W. S., 1952, Thyroid function in some anadromous and landlocked teleosts. Trans. R. Soc. Canada. Sect. 5. 46: 39–53.

Hogben, L., 1929, The comparative physiology of internal secretion. Cambridge Univ. Press.

Holmgren, N., 1939, Contribution to the question of the origin of the tetrapod limb. Acta Zool. 20: 89–124.

Hopper, A. F., 1952, Growth and maturation response of *Lebistes reticulatus* to treatment with mammalian thyroid powder and thiouracil. J. exp. Zool. 119: 205–219.

Hoyer, H., 1934, Das Lymphgefässsystem der Wirbeltiere vom Standpunkte der vergleichenden Anatomie. Memoires Acad. Polon. Sci. Cl. Méd. 1. Cracovie.

Huxley, J. S., 1923, Time relations in amphibian metamorphosis. Sci. Progr. 17: 606 (Quoted after de Beer 1940).

Ieremieieva, E. F., 1948, Stroienie i razvitie rotovogo apparata leshtsha, volby i sazana. Sbornik Morph. osob. opried. pitan. etc. Ak. N. .USSR. :54–143.

Irikhimovitsh, A. J., 1948 a, Razvitie gipofiza i shtshitovidnoi shelezy leshtsha i sievrugi. Dokl. A. N. USSR. 60: 135–136.

1948 b, Sravnitelnoie issledovanie litshinotshnogo razvitia ryb i amphibii v sviazi z razvitiem i funkciei gipophizarno-tireoidnogo komplexa. Dokl. A. N. USSR. 60: 1101–1104.

1950, Issledovanie faz nakoplenia i viviedienia gormona shtshitovidnoi shelezy u molodi osietrowykh ryb. Dokl. A. N. USSR. 73: 857–860.

Jurand, A., 1955, Zjawisko neotenii u *Xenopus laevis.* Folia Biol. 3: 315–330.

Klumpp, W., und Eggert, B., 1935, Die Schilddrüse und die branchiogenen Organe von *Ichthyophis glutinosus* L. Z. wiss. Zool. 146: 329–381.

Koch, H. J., et Heuts, M. J., 1942, Influence de l'hormone thyroïdienne sur la régulation osmotique chez *Gasterosteus aculeatus* L. forme gymnurus Cuv. Ann. Soc. R. Zool. Belg. 73: 165–172.

Kotthaus, A., 1933, Die Entwicklung des primordial-Cranium von *Xenopus laevis* bis zur Metamorphose. Z. wiss. Zool. 144: 510–572.

Kratochwill, K., 1933, Zur Morphologie und Physiologie der Nahrungsaufnahme der Froschlarven. Z. wiss. Zool. 144: 421–468.

Kryshanovski, S. G., 1933, Organy dykhania litshinok ryb (Teleostomi) i pseudobrankhia. Tr. Labor. Evol. Morphol. A. N. USSR. 1.

La Roche, G., 1950, Analysis of the effects of thyroid preparations on the young river salmon (parr). Anat. Rec. 106: 213.

La Roche, G., Leblond, C. P., et Préfontaine, G., 1950, Effects de l'hormone thyroïdienne sur le saumon de l'Atlantique: stade parr. Rev. Canad. Biol. 9: 101–103.

Leloup, J., 1947, Action des antithyroïdiens (aminothiazol et phénylthiourée) sur la cuprémie dans la série des Vertébrés. Bull. Soc. Chim. Biol. 29: 582–595.

1948, Influence d'un abaissement de salinité sur la cuprémie de deux Téléostéens marins: *Muraena helena* L., *Labrus bergylta* Asc. C. R. Soc. Biol. 142: 178–179.

1949, L'iodémie des Téléostéens marins. C. R. Soc. Biol. 143: 330–332.

Lynn, W. G., 1948, The effects of thiourea and phenylthiourea upon the development of *Eleutherodactylus ricordii.* Biol. Bull. 94: 1–15.

Lynn, W. G., and Lutz, B., 1946, The development of *Eleutherodactylus nasutus* Lutz (Salientia). Bol. Mus. Nation. Rio de Janeiro. 79: 1–30.

Lynn, W. G., and Peadon, A. M., 1955, The role of the thyroid gland in direct development in the anuran *Eleutherodactylus martinicensis*. Growth 19: 263–286.

Lange, O. N., 1950, Stroienie i razvitie kishetshnika niekotorykh viunowykh. Tr. Inst. Morph. Shiv. A. N. USSR. 3: 87–109.

Murr, E., und Sklower, A., 1928, Untersuchungen über die inkretorischen Organe der Fische. I. Das Verhalten der Schilddrüse in der Metamorphose des Aales. Z. vergl. Physiol. 7: 279 (Quoted after Hagen 1936).

Noble, K. G., 1931, The biology of the amphibia. McGraw-Hill. New York and London.

Olifan, V. J., 1945, Periodicity in the development and critical stages of the early postembryonic ontogenesis of *Acipenser stellatus*. Bull. Acad. Sci. USSR. 1945: 56–78.

Olivereau, M., 1948, Influence d'une diminution de la salinité sur l'activité de la glande thyroïde chez deux Téléostéens marins: *Muraena helena* L., *Labrus bergylta* Asc. C. R. Soc. Biol. 142: 176–177.

1949 a, L'activité thyroïdienne chez *Torpedo marmorata* Riss. au cours du cycle sexuel. C. R. Soc. Biol. 143: 212–214.

1949 b, L'activité thyroïdienne de *Scyllium canicula* L. au cours du cycle sexuel. C. R. Soc. Biol. 143: 247–250.

1950, Influence d'une augmentation de salinité sur l'activité thyroïdienne de divers Téléostéens d'eau douce. C. R. Soc. Biol. 144: 775–776.

1954, Hypophyse et glande thyroïde chez les poissons. Ann. Inst. Océanogr. 29: 95–296.

Olko, A., 1955, The respiratory vessels of the yolk-sac of the trout, *Salmo trutta* L. Zoologica Polon. 6: 216–225.

Portmann, A., 1935, Die Ontogenese der Vögel als Evolutions-problem. Acta Biotheor. 1: 57–90.

Robertson, O. R., 1948, The occurrence of increased activity of the thyroid gland in rainbow trout at the time of transformation from parr to silvery smolt. Physiol. Zool. 21: 282–295.

Sachs, M. G., and Zamkova, M. A., 1947, On the role of the thyroid in the embryogenesis of vertebrates. J. Physiol. USSR. 33: 449–462.

Savage, R. M., 1952, Ecological, physiological and anatomical observations on some species of anuran tadpoles. Proc. Zool. Soc. London. 122: 467–514.

1955, The ingestive, digestive and respiratory systems of the microhylid tadpole, *Hypopachus aguae*. Copeia 1955: 120–127.

Schulze, F. E., 1892, Über die inneren Kiemen der Batrachierlarven. Abh. Akad. Wiss. Berlin. 1892/3: 1–66.

Scott, J. L., 1953, The effects of thiourea treatment upon the thyroid, pituitary and gonards of the zebra fish, *Brachydanio rerio*. Zoologica (N. Y.) 38: 53–62.

Sedra, S. N., 1950, The metamorphosis of the jaws and their muscles in the toad, *Bufo regularis* Reuss, correlated with changes in the animal feeding habits. Proc. Zool. Soc. London. 120: 405–449.

Sembrat, K., 1929, The influence of fish thyroid transplants on the metamorphosis of *Rana temporaria* L. larvae and on their thyroid and thymus glands. Experiments with thyroid glands of Selachians (*Scyllium canicula* Cuv. and *Scyllium stellare* Gthr.) and Teleosts (*Cyprinus carpio* L.). Bull. Acad. Polon. Sci. Sér. B. 1928: 461–488.

1953, Effects of the endostyle of the lancelet (*Branchiostoma lanceolatum* Pall.) on the metamorphosis of axolotl. Zoologica Polon. 6: 3–19.

1954, Wpływ tarczycy na skórę troci (Salmo trutta L.). Kosmos MA. 3: 103.

1956, Influence of the thyroid gland on the skin of Teleosts. Zoologica Polon. 7: 3–24.

Severtzoff, A. N., 1927, Über die Beziehungen zwischen der Ontogenese und der Phylogenese der Tiere. Jen. Z. Naturwiss. 63: 51 (Quoted after Russian ed. Moskow 1945).

Skadovski, C. N., 1949, Perspektivy osietrovodstva po dannym ekologo-fiziologitsheskogo izutshienia kurinskoi sievrugi. Rybn. Khoz. 1949/2: 39–44.

Spurway, H., 1953, Genetics of specific and subspecific differences in European newts. In "Evolution", Symp. Soc. Exp. Biol. 7: 200–237.

Stefanelli, A., 1953, Il neurone di Mauthner degli Ittiopsidi (Pisces, Amphibia). Experientia 9: 277–285.

Stephenson, N. G., 1951 a, On the development of chondrocranium and visceral arches of *Leiopelma archeyi.* Trans. Zool. Soc. London. 27: 203–253.

1951 b, Observations on the development of the amphicoelus frogs, Leiopelma and Ascaphus. Linn. Soc. J. Zool. 42: 18–28.

Stolk, A., 1951, Activity of the embryonic thyroid gland in the viviparous cyprinodont *Lebistes reticulatus* Peters. K. Nederl. Ak. Wetensch. Amsterd. Proc. 54: 579–584.

Strawiński, S., 1956, The vascularization of the respiratory surfaces of the edible frog (*Rana esculenta* L.) during ontogenesis. Zoologica Polon. 7 (in print).

Szarski, H., 1956, Causes of the absence of a stomach in Cyprinidae. Bull. Acad. Polon. Sci. Cl. II. 4: 155–156.

Szarski, H., Delewska, E., Leja, S., Olechnowiczowa, S., Predygier, Z., and Siankowa, L., 1956, The digestive system of the bream (*Abramis brama* L.). Studia Soc. Sci. Tourunensis. Sect. E. 3: 113–146.

Schmalhausen, I. I., 1954, Razvitie shabier, ikh krovienosnykh sosudov i muskulatury u amfibii. Zool. Shurn. 33: 848–868.

Underhay, E., and Baldwin, E., 1955, Nitrogen excretion in the tadpoles of *Xenopus laevis.* Bioch. J. 61: 544–547.

Vasnietzov, V. V., 1948, Etapy razvitia sistemy organov, sviazannykh s pitaniem, u leshtsha, volby i sazana. Sbornik Morph. osob. opied. pitan. etc. A. N. USSR: 233–263.

Vernidub, M. F., and Guzieva, M. I., 1950, O morfologitsheskikh etapakh v razvitii litshinok ryb. Dokl. A. N. USSR. 71: 585–588.

Vilter, V., 1945 a, Inhibition expérimentale de la croissance stomachale au cours de la métamorphose larvaire de l'anguille. C. R. Soc. Biol. 139: 817–818.

1945 b, Hypophysectomie chez la larve d'anguille (civelle) et repercussions sur la métamorphose pigmentaire. C. R. Soc. Biol. 139: 816–817.

Vrtel, S., 1931, Histologische Untersuchungen über die Schilddrüse. I. Die Selachierschilddrüse. Bull. Acad. Polon. Sci. Sér. B. II. 1931: 155–200.

Weiss, P., 1939, Principles of development. A text in experimental embryology. Henry Holt. New York.

Witschi, E., 1955, Adaptation de l'oreille de l'anoure a la vie aquatique et en surface. VI Congr. Féd. Int. Anat. Paris. Masson. :229–230.

EMBRYONIC TEMPERATURE ADAPTATIONS IN HIGHLAND *RANA PIPIENS*[1]

E. PETER VOLPE

Newcomb College of Tulane University, New Orleans, Louisiana

INTRODUCTION

Embryos of leopard frogs from northern localities (Vermont, Wisconsin, New Jersey, and Quebec) have a lower range of temperature tolerance and a more rapid rate of development at low temperatures than those from southern areas (Louisiana, Florida, Texas, and eastern Mexico) tested under the same experimental conditions (Moore, 1949). Moreover, interpopulation hybrids exhibit progressively greater developmental defects as the north-south distance between the sources of the parents increases (Moore, 1946, 1947). The most severe abnormalities, resulting in the death of most or all of the embryos, occur in hybrids between *Rana pipiens* from Vermont (or Wisconsin) and eastern lowland Mexico (Moore, 1947; Volpe, 1954; Ruibal, 1955).

If these findings are correlated, as they appear to be, with regional differences in environmental temperatures, then leopard frogs from high altitudes, irrespective of latitude, would be expected to possess embryonic temperature characteristics that resemble those of individuals from northern localities. Indeed, crosses between female leopard frogs from the Lake Champlain region of Vermont and males from an altitude of 3,700 feet in Moravia, Costa Rica, produce viable hybrids that exhibit only slight defects (Moore, 1950). The reverse cross, Costa Rica ♀ × Vermont ♂, was not performed. Later studies by Ruibal (1955) revealed that reciprocal crosses between *R. pipiens* from Vermont and Zempoala (9,840 feet) in the Sierra de Ajusco of Mexico yield also essentially normal embryos.

However, the climate in the central highlands (3,000–5,000 feet) of Costa Rica is not typical of elevated regions. The climate at Moravia is temperate, lacking the seasonal changes that occur in the *sierra madres* of Mexico or the northern part of the United States (fig. 1*A*). Moore (1950) found egg masses of *R. pipiens* in pasture washes in the finca of Don Fernando Alvarado-Chacun (now owned by George H. Harvey) at Moravia on July 3, 1949. I found leopard frogs in breeding activity in the same ranch on September 10 and 11, 1956. It is probable that the frogs breed throughout the year at this locality. (Residents of the finca informed me that choruses of

[1]This investigation was supported by a grant from the National Science Foundation (NSF-G3332). Expenses of the flight to Costa Rica were defrayed by funds from the Tulane University Council on Research.

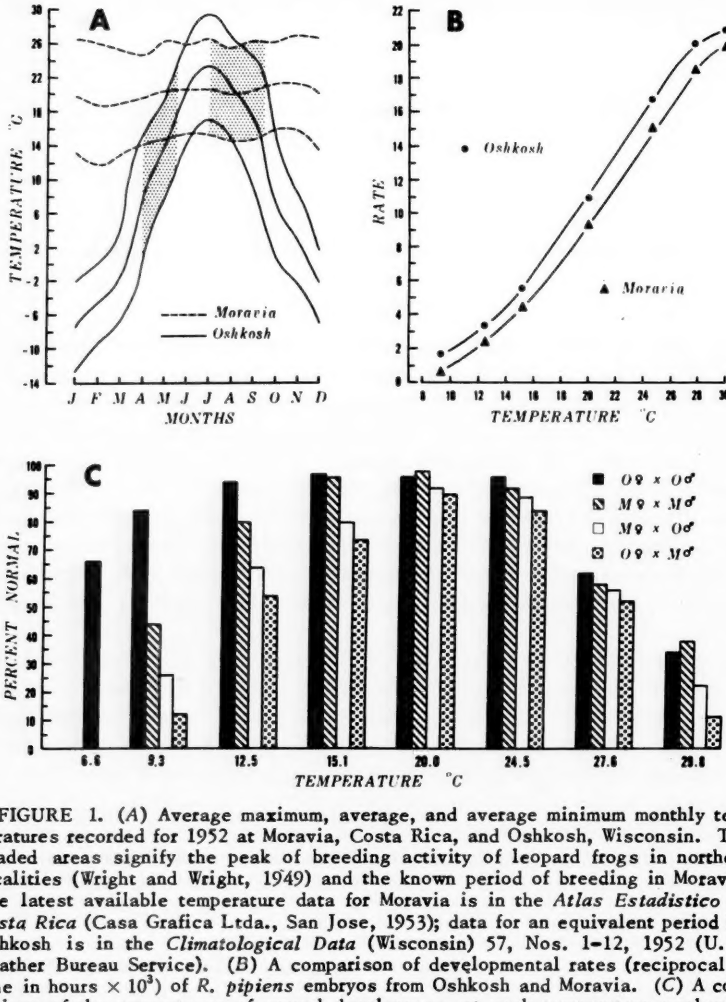

FIGURE 1. (*A*) Average maximum, average, and average minimum monthly temperatures recorded for 1952 at Moravia, Costa Rica, and Oshkosh, Wisconsin. The shaded areas signify the peak of breeding activity of leopard frogs in northern localities (Wright and Wright, 1949) and the known period of breeding in Moravia. The latest available temperature data for Moravia is in the *Atlas Estadistico de Costa Rica* (Casa Grafica Ltda., San Jose, 1953); data for an equivalent period for Oshkosh is in the *Climatological Data* (Wisconsin) 57, Nos. 1–12, 1952 (U. S. Weather Bureau Service). (*B*) A comparison of developmental rates (reciprocal of time in hours $\times 10^3$) of *R. pipiens* embryos from Oshkosh and Moravia. (*C*) A comparison of the percentages of normal development, at each temperature employed, of embryos of leopard frogs from Oshkosh and Moravia, and of reciprocal hybrids.

R. pipiens may be heard, and newly transformed young may be seen, at all times of the year.) Considering the mild, non-fluctuating climate at Moravia, one would surmise that the leopard frogs of this region would possess different temperature-related characteristics than those from colder habitats. On the other hand, in view of Moore's finding of relatively good hybrid compatibility between Vermont and Moravia leopard frogs, the temperature adaptations of Moravia embryos should be similar, in some aspect, to those of Vermont (or Wisconsin) embryos.

The present investigation was designed to ascertain the rates of development and limits of temperature tolerance of embryos of *R. pipiens* from Moravia, as well as to obtain supplementary data on hybridization between Moravia and northern leopard frogs. The latter were derived from the vicinity of Oshkosh, Wisconsin.

METHODS

Five series of artificial fertilizations were performed; each series consisted of the following crosses: Moravia ♀ × Moravia ♂, Moravia ♀ × Oshkosh ♂, Oshkosh ♀ × Oshkosh ♂, and Oshkosh ♀ × Moravia ♂. Ovulation was induced by the standard pituitary injection method; the eggs were stripped from the gravid females into sperm suspensions. The four groups of fertilized eggs of each series were placed in individual finger bowls containing 200 cc. of 0.10 Ringer's solution. Approximately 75 eggs were kept in each bowl. Prior to first cleavage of the egg, the bowls were distributed among constant-temperature units, which maintained temperatures accurate to ± 0.2° C. Eggs of the first series were kept at 20.0°, 27.6° and 29.8°; of the second series, at 12.5°, 15.1° and 20.0°; of the third series, at 20.0°, 24.5° and 27.6°; of the fourth series, at 9.3°, 20.0° and 29.8°; and of the fifth series, at 6.6°, 9.3° and 20.0°.

The embryos at each temperature were examined at close intervals during their development from first cleavage (stage 3) to a chosen end-point, gill circulation (stage 20). The stages of development are defined in Pollister and Moore (1937).

Some of the embryos of the first series of crosses were reared through metamorphosis. After the embryos in the 20° temperature cabinet had attained stage 20, approximately 15 from each cross of the series were placed in pond water in 12 by 8 by 2-inch white enamel pans. The pans were kept in an air-conditioned laboratory, in which the temperature fluctuated from 17° to 23° C. The tadpoles were fed boiled spinach. When the tadpoles approached metamorphosis, the water level in the pans was reduced to enable the young frogs to emerge from the water.

RESULTS

At each temperature tested, the Oshkosh embryos (Oshkosh × Oshkosh) reached the experimental end-point, stage 20, in significantly less time than that taken by the Moravia embryos (Moravia × Moravia). At 20.0° C., the Oshkosh embryos attained stage 20 in 92.8 hours (average of five experiments; range: 91.5–95.7 hours), as compared to 105.5 hours (range: 103.5–108.5 hours) required by the Moravia embryos. The complete data of one experiment at 20.0° are given in table 1.

The developmental differences were greater at lower temperatures. At 15.1° and 12.5°, the time intervals between first cleavage and gill circulation for the Oshkosh embryos were 183 and 305 hours respectively; for the Moravia embryos, 219.5 and 365 hours respectively. The relationship at all temperatures is shown in fig. 1*B*, where the reciprocal of time between

TABLE 1

STAGES IN THE EMBRYONIC DEVELOPMENT AT 20.0° C. OF *RANA PIPIENS* FROM MORAVIA, COSTA RICA AND OSHKOSH, WISCONSIN, AND OF THEIR RECIPROCAL HYBRIDS

Age in Hours	Moravia ♀ × Moravia ♂ (73)*		Moravia ♀ × Oshkosh ♂ (76)	Oshkosh ♀ × Oshkosh ♂ (69)		Oshkosh ♀ × Moravia ♂ (72)
0	3**		3	3		3
2.5	5	=***	5	5	=	5
7	7	=	7	7	=	7
16.7	9	=	9	9	=	9
20	10E****	=	10E	10E	=	10E
24.2	11E	=	11E	11E	=	11E
27	12E	=	12E	12E	=	12E
31.2	12M	=	12M	12L	> ?	12M-L
41	13E-M	>	13E	13M	>	13E
46.5	14E	>	13L	14L	>	14E
52.2	14L	>	14E	16E	>	15
64	16L	>	16M	17L	>	17E
70	17E-M	>	17E	18E	>	17M-L
76.7	17L	>	17M	19E	>	18M
89	18E	>	17L	19L	>	19E
93.2	18L	>	18E	20E	>	19L
96	19E	>	18L	-		20E
101	19L	>	19M	-		-
105	20E	>	19L	-		-
113	-		20M	-		-

*Indicates the number of embryos in the group examined.

**The embryos were considered to be in a given stage when 50 percent or more exhibited the characteristics for that stage.

***An " = " sign between two figures indicates that the embryos being compared were indistinguishable in their development. A " > " indicates that the em- in the left column were more advanced.

****The stages are described in Pollister and Moore (1937); the letters "E", "M", and "L" signify early, middle, and late respectively.

stages 3 and 20 is taken as the measure of developmental rate and plotted against temperature.

Metamorphosis occurred at an earlier date in the Oshkosh than in the Moravia larvae reared under the same laboratory conditions. Eight Oshkosh tadpoles metamorphosed within a period of 89-96 days (after fertilization), whereas six Moravia tadpoles transformed within 119-127 days.

The percentages of the Oshkosh and Moravia embryos that developed normally at each of the experimental temperatures are indicated in fig. 1*C*. As in previous studies, a temperature at which 50 percent or more of the embryos were inviable constituted a lethal temperature. The responses of the Oshkosh and Moravia embryos to high temperatures were similar. The 50 percent level of inviability lies between 27.6° and 29.8°.

The Moravia embryos were more susceptible to low temperatures than were the Oshkosh embryos. The latter tolerated temperatures as low as 6.6°. The blastopore lips were irregular in the Moravia embryos reared at 6.6°. All embryos cytolyzed in the late gastrula stage. At 9.3°, the

Moravia embryos were normal in the early stages of development; after hatching, more than 50 percent of the embryos failed to initiate heart beat or establish gill circulation. The lower limiting temperature for normal development of Moravia embryos is between 9.3° and 12.5°, closer to the former value.

Both types of hybrids (Oshkosh ♀ × Moravia ♂ and Moravia ♀ × Oshkosh ♂) developed more slowly than their controls (Oshkosh ♀ × Oshkosh ♂ and Moravia ♀ × Moravia ♂ respectively). The deviations from the maternal rates in each type of hybrid were invariably evident in the neural plate stage (stage 13). In some experiments, as indicated for one experiment in table 1, the retardation in hybrid rate of development appeared to begin at the close of gastrulation (stage 12). The retarded rates were associated with specific developmental irregularities. The neural folds in the anterior region of the Oshkosh ♀ × Moravia ♂ embryos were more divergent than in the control embryos, whereas they were closer together in the reciprocal hybrid embryos. These effects were reflected in later development by an enlargement of the head region in the Oshkosh ♀ × Moravia ♂ embryos and a reduction of head structures in the Moravia ♀ × Oshkosh ♂ embryos. At 24.5° and 20.0°, the head region was affected slightly and most of the embryos were viable at stage 20.

The hybrid defects were expressed to a greater degree at low temperatures (fig. 1*C*). At 15.1° and, more strikingly, at 12.5°, the hybrid embryos exhibited typical syndromes associated with macrocephaly and microcephaly. Inviable embryos of the Oshkosh ♀ × Moravia ♂ cross had enlarged heads, widely-separated oral suckers, shortened bodies and poorly-differentiated gills; those of the reciprocal cross had small heads, fused suckers and olfactory pits, edematous abdomens, and distorted tails. The significant point, however, is that the majority of the hybrid embryos were viable at all temperatures within normal tolerance limits of the parental embryos, a situation in marked contrast to other crosses between northern and southern leopard frogs (e.g., Vermont × Texas, Wisconsin × lowland Mexico, and Vermont × lowland Mexico). In the latter crosses, the hybrid embryos possess the same anomalies stated above but to such a severe degree that most or all of the embryos die prior to, or shortly after, hatching. Moreover, the Moravia × Oshkosh hybrids can be reared successfully through metamorphosis. Under laboratory conditions, five Oshkosh ♀ × Moravia ♂ tadpoles transformed within a period of 124–129 days (after fertilization); four Moravia ♀ × Oshkosh ♂ tadpoles metamorphosed within 137–143 days.

DISCUSSION

Embryos of leopard frogs from Moravia, Costa Rica, possess features that combine the characteristics of northern and southern embryos of *R. pipiens* as well as those that are of adaptive value to their unique environment. Embryos from southern localities are able to tolerate temperatures above 30° C.; the upper limiting temperature for normal development ranges

from 32° for Texas and Louisiana embryos to 35° for southern Florida embryos (Moore, 1949). Such temperatures are lethal for Moravia and northern embryos. The upper limit of temperature tolerance of embryos from Vermont, Wisconsin, New Jersey, and Quebec is 28° (Moore, *op. cit.*). The Moravia embryos resemble those of most southern leopard frogs in being susceptible to temperatures below 9°. The lower limit of temperature tolerance of Vermont or New Jersey embryos is 5° (Moore, *op. cit.*); embryos from Wisconsin tolerate temperatures at least as low as 6.6°.

The Moravia embryos have a narrow temperature tolerance range, between 9.3° and 12.5° at one end of the temperature scale and between 27.6° and 29.8° at the other end (fig. 1*C*). This apparently is correlated with the small variations in environmental temperatures at Moravia (fig. 1*A*).

The slow rate of development of Moravia embryos may be causally associated with a long growing season. Ordinarily the length of the growing season becomes progressively shorter as the altitude or latitude increases, and most cold-adapted ranid embryos possess a rapid developmental rate. It would appear that the year-round temperate condition at Moravia precludes selection for a rapid developmental rate in the leopard frogs of this region.

The Moravia and Oshkosh embryos have similar tolerance values in the upper range of temperatures (fig. 1*C*). The responses of the reciprocal hybrid embryos to high temperatures correspond to those of the parental embryos. The defects in the hybrid embryos are slight at 24.5° and 20.0°, but become progressively more pronounced as the temperature is lowered. It is to be noted also that the temperature tolerance values of the parental embryos become more divergent with decreasing temperatures. In essence, the intensity of hybrid defects at low temperatures parallels the magnitude of the parental differences in embryonic temperature tolerance. The greater irregularities in hybrid development at lower temperatures may be attributable, at least in part, to conflicting modes of action of temperature-related processes.

Whatever may be the underlying factor(s) responsible for the hybrid abnormalities, the fact remains, confirming Moore's finding (1950), that hybids between leopard frogs from the central highlands of Costa Rica and northern localities in the United States are more viable than hybrids from certain other crosses of northern and southern *R. pipiens* (described in Moore, 1946, 1947; Volpe, 1954, Ruibal, 1955).

SUMMARY

The relatively high degree of hybrid compatibility between leopard frogs from an altitude of 3,700 feet at Moravia, Costa Rica and northern localities (Vermont or Wisconsin), demonstrated by Moore (1950) and confirmed herein, prompted the inference that the Moravia leopard frogs are cold-adapted, like northern leopard frogs. On the contrary, a study of certain embryological characteristics of leopard frogs from Moravia, Costa Rica and Oshkosh, Wisconsin has revealed that embryos of the former have a slower rate of development at any given temperature, are less resistant to low tempera-

tures, and have a narrower range of temperature tolerance than embryos of the latter. However, the Moravia and Oshkosh embryos have similar tolerances to high temperatures. It has been shown also that the defects in the hybrid embryos are greater at low than at high temperatures, but they are not as severe as those observed previously in hybrid embryos from other crosses of northern and southern *R. Pipiens* (e.g., Vermont × Texas, Wisconsin × lowland Mexico, and Vermont × lowland Mexico).

LITERATURE CITED

Pollister, A. W., and J. A. Moore, 1937, Tables for the normal development of *Rana sylvatica*. Anat. Rec. 68: 489–496.

Moore, J. A., 1946, Incipient intraspecific isolating mechanisms in *Rana pipiens.* Genetics 31: 304–326.

1947, Hybridization between *Rana pipiens* from Vermont and eastern Mexico. Proc. Nat. Acad. Sci. 33:72–75.

1949, Geographic variation of adaptive characters in *Rana pipiens* Schreber. Evolution 3:1–24.

1950, Further studies on *Rana pipiens* racial hybrids. Amer. Nat. 84:247–254.

Ruibal, R., 1955, A study of altitudinal races in *Rana pipiens*. Evolution 9:322–338.

Volpe, E. P., 1954, Hybrid inviability between *Rana pipiens* from Wisconsin and Mexico. Tulane Stud. Zool. 1:111–123.

Wright, A. H., and A. A. Wright, 1949, Handbook of frogs and toads of the United States and Canada. Comstock Publishing Company, New York.

Vol. XCI, No. 860 The American Naturalist September–October, 1957

ON THE ORIGIN OF MATING BEHAVIOR IN SPIDERS

ANNE J. ALEXANDER AND D. W. EWER

Department of Zoology, Rhodes University, Grahamstown, South Africa

> **"It is difficult, ... to give a plausible explanation of the origin of the type of mating characteristic of spiders."**
>
> **Petrunkevitch, (1952).**

Over the last few years some interest has been focussed on the question of insemination in the scorpion, as shown in papers by Piza (1950), Vachon (1953), Baerg (1954), Southcott (1955) and Shulov (1956). Recently, Angerman (1955) has given the first account of the actual mating of these animals ana what he has established for the Chactidae has been observed in this laboratory for the Scorpionidae and Buthidae (Alexander, 1956 and 1957) while Zolessi (1956) reports the same behavior for the Bothriuridae. In all cases the male deposits a fairly substantial spermatophore on the ground and the female receives the sperm from that part of the structure which enters her genital opening. This resolution of the problem of scorpion mating allows arachnid mating habits to be seen as a whole for there are now only a few groups in which these are totally unknown. In addition it has given rise to a renewal of interest of the origins of such behavior. This present paper on spider mating does not offer any new information on the details of sexual behavior in spiders but is a re-appraisal of the known facts in the light of the recent observations on other arachnids.

FERTILIZATION IN SPIDERS

A male spider, shortly after he reaches maturity, deposits a drop of semen either on the web on which he lives, on that of his mate or on one especially constructed for the purpose. Dipping his palps alternately into this drop, he draws the sperm into the swollen tips of these appendages. This is the process of sperm induction, first described by Menge in 1843. In some cases this sperm induction occurs only in the presence of the female, (Bristowe & Locket, 1926) while in others (eg. the tarantulas of Baerg, 1928) the female need not necessarily be there. Later the male advances towards the female with the courtship characteristic of the species, or the female may make the advances, as in the cases reported by Montgomery (1903) and Locket (1926). He mounts her, again in a manner characteristic of the species, and inserts first one, then the other palpal tip into her genital opening, although, in certain rare cases, both palps are inserted together. During these insertions the sperm are transferred into the female. In most spiders sperm induction and actual mating occur only once during the life of the male, (Petrunkevitch, 1952) but in some, for ex-

ample, the tarantulas, they may occur as many as four times, each mating being preceded by the process of sperm induction.

Most workers on the subject would agree with Montgomery (1903) that there is "neither anatomical nor embryological reason for supposing that the palpi ever had been appendages of the genital aperture." He comments on the peculiarity of the "double process" of spider mating—the sperm induction and then the actual transference of the sperm to the female—and makes two suggestions as to how the phenomenon could have arisen.

If the pedipalps were originally used as clasping organs during a copulation in which the genital apertures of the pairing animals were apposed, the depression in the epigynum of the female might have evolved to-correspond to projections upon the palpal organs of the male and thus led to the elaboration of a tube within each of the palpal organs; thus the sperm induction and the actual mating could be dissociated.

Alternatively, he suggests the palps might have been used originally to carry drops of semen from the male to the female genital opening and later, when the appropriate apparatus for the storage of sperm had been evolved, the male would discharge semen onto the web, take it into his palps and later pass it into the female with these organs.

Both of these hypotheses do in fact allow the possible selective advantages of the behavior to be recognised: it permits the male to prepare for the mating beforehand and, when in the proximity of the female, to be as quick as possible with the actual insemination. Where the female is very often bigger than the male, where the animals are carnivorous, preying on arthropods more or less their own size, and where there is no development of social instincts which would check cannibalistic tendencies, it would certainly be safer for the male to remain with the female for as short a time as possible when mating.

The difficulty is that neither of these hypotheses makes any attempt to explain the "double process" which is the basis of the problem. The former shows only how the pedipalps might have been elaborated to form a "sperm-gun"; the latter assumes that the pedipalps played this role from the beginning. Neither of them offers any explanation of the origin of a sperm induction where the sperm are first shed *externally* before being introduced into the female. Indeed both explanations would lead one to expect that the male would take the sperm directly from his genital opening into his pedipalps. In point of fact, Petrunkevitch (1952) states that the male spider cannot reach his genital orifice with his palps.

The only other solution offered is that of Bristowe (1929) and this is, in the main, merely an elaboration of the second hypothesis of Montgomery. Bristowe says:—"in the primitive arachnid the chelicerae were chelate, and used by the male to hold the sperm after it had been ejaculated and to place it in the female vulva. Then at a later stage, the sperm was picked up by one of the longer pairs of appendages and transmitted to the female by them. In course of time the appendages used for this purpose became specially modified, and small cavities appeared at

the tip of the palpi to hold the sperm." Here too the origin of the sperm-induction has merely been side-stepped.

In the hypothesis put forward here some attempt is made to explain this very point. Since the explanation offered arises from a comparative survey of all known arachnid mating behavior, it is intended to start by considering a hypothetical protoarachnid and its possible manner of fertilization.

FERTILIZATION IN A PROTOARACHNID

The relation of the Xiphosura to the true arachnids is still unclear but from the time of the early anatomical work of Lankester (1881) to the serology and heart physiology of recent times (Leone, 1954 and Sawaya & Soares, 1949) it has been apparent that the relationship is fairly close. It is well therefore to consider the mating behavior of the king-crab. It is very simple: the male clings to the back of the female and when she lays her eggs he covers them with sperm, (Shuster, 1950). Quite apart from the possible relationship of the Xiphosura to the true arachnids, this is the sort of mating behavior which might well be expected in a protoarachnid, in that it is behavior normal amongst aquatic animals and it is commonly agreed that the arachnids must have arisen from some such beast.

Once the arachnids became truely terrestrial, internal fertilization became highly desirable because of dangers of desiccation of the genital products. Furthermore, internal development of the embryo, which probably evolved in many forms as a protection against desiccation, clearly demands internal fertilization. Certainly some form of internal insemination is one of the prerequisites for terrestrial life in general. In the scorpions, pseudo-scorpions, solifuges and certainly in some ticks and mites the difficulty was overcome in the following manner: the male still simply extrudes the sperm but a spermatophore is elaborated about it so that a variety of methods become available for its transfer into the female. The problem of the origin of this behavior will not be considered here but clearly studies on the evolution of similar habits in newts where transfer of a spermatophore has evolved from a simple amplexus, may be relevant. (vide Noble 1931).

However, where the male is the same size as the female throughout the group, where there is some social instinct developed, as in harvestmen which tend to be gregarious: (Savory, private communication and personal observation), where the animals are parasitic (e.g. ticks and some mites) or where they are phytophagous (e.g. some mites) direct insemination could be evolved. The genital opening of one animal could be applied to that of the other and, as has occurred in the phalangids, intromittent organs associated with the genital segment could have evolved. It is not clear whether such a line of evolution could have allowed within it the development of intromittent organs from structures merely associated closely with the genital region, such as the mouthparts of the ticks. It seems however, more probable that, at least in the case of both the ticks and the mites,

the rather diverse mating habits reflect an origin from behavior akin to that of the scorpions. The report of Byers *et al* (1957) of indirect transference of a spermatophore in trombiculid mites and that of Nutall and Merriman (1911) on *Ornithodorus* support this strongly.

In the case of the spiders, two interpretations of the behavior may be postulated: either the animals have retained the Limulus-like habit of depositing sperm on the substratum but have further evolved the elaborate palpal "pick-up" system or they have gone through a stage of mating like that of the modern scorpions and pseudo-scorpions but have lost completely the spermatophore covering of the sperm mass. These two possible lines of evolution are shown diagrammatically in fig. 1.

The first suggestion does not seem very probable if we are considering the evolutionary path of the actual behavior. Clearly internal fertilization could not be achieved until the pedipalpar mechanism was developed; at the same time there does not seem any obvious reason why the male should pick up the sperm while fertilization is still external. The shortcomings of the hypothesis arise from the fact that it requires the simultaneous development of the palpal appendages, or more important, the male behavior pattern, and the female habit of retaining the eggs inside her before fertilization.

Consider the second possibility, namely that the spiders have passed through a stage in which they deposited a spermatophore on the ground, this being picked up by the female. That is, the sperm deposition of modern spiders is a phylogenetic vestige of the "spermatophore-depositing" behavior of the proto-spiders. It is desirable at this point to look briefly at the various methods by which the spermatophore of an arachnid may be transferred to the female from the ground where the male has deposited it. This is achieved in a number of different ways.

METHODS OF SPERMATOPHORE TRANSFERENCE

In the scorpions (Angerman, 1955, Alexander 1956 and Zolessi, 1956) and some of the pseudoscorpions (Kew, 1912 and Vachon, 1938) it is done without the direct use of any of the appendages of the male or the female, so that either the co-operation between the two animals or the structure of the spermatophore or both have been greatly elaborated to ensure an efficient mating. In other arachnids various limbs of the male are used in moving the sperm into the female. In the pseudoscorpion, *Chelifer latreilli* Leach, Kew (1912) describes how the male holds open the female genital orifice with the modified claws of his first legs while he eases her onto the naked sperm mass on top of the spermatophore. In the ricinulids, Comstock (1940) considers that the third leg is involved in the transference of the spermatophore to the female. In the solfugids the chelicerae are used for moving the "Spermaballen" from the ground where the males deposit them to the genital openings of the females (Heymons, 1901).

It is thus clear that the spermatophores or "Spermaballen" may be transferred to the female by the chelicerae, first legs, and third legs.

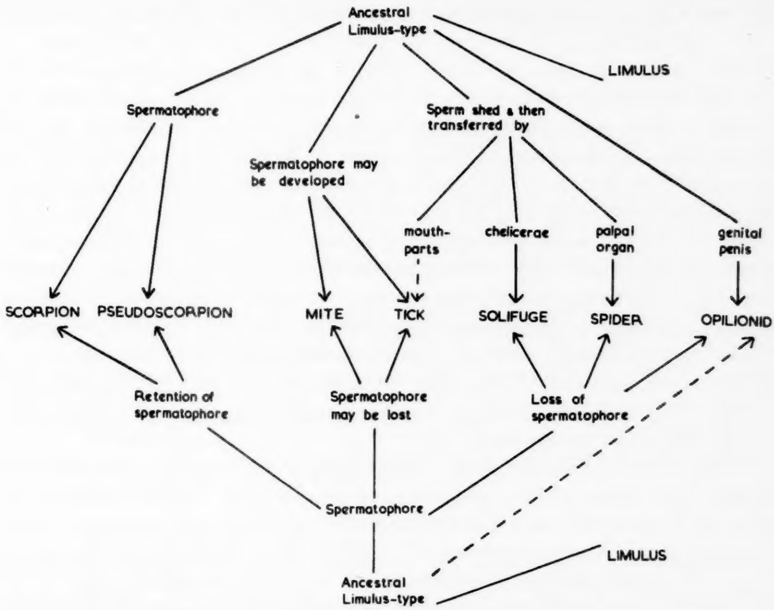

FIGURE 1.

There thus seems no obvious objection to the postulate that the primitive spiders used their pedipalps in transferring the sperm from the deposited spermatophore into the female, or, alternatively, that the palps were used for holding open the genital aperture, as are the legs of the pseudoscorpion mentioned above. It is possible that initially there was a tendency to use the chelicerae as well as or instead of the pedipalps and thus Bristowe's suggestion may be included within the limits of the present hypothesis.

The question of the loss of the spermatophore can be considered quite separately, beginning once more with the primitive arachnid. A mass of sperm merely deposited on the ground is likely to be absorbed immediately between the soil particles, a factor which may well have influenced the evolution of the spermatophore in the scorpions and pseudoscorpions. Even when the problem of the protection of the sperm from being absorbed into the soil has been solved by the elaboration of a spermatophore, the availability of a surface suitable for mating remains of great importance. In the scorpions mating cannot take place if the surface on which the animals are placed is not hard enough to support the spermatophore (Alexander, 1957). The fact that spiders will normally either meet on a web or can spin one when it is needed, must give them a significant advantage as far as mating is concerned. This may well be one of the reasons for the success of this arachnid group relative to the others.

The pseudoscorpion, C. *cancroides* L., has a spermatophore which is an almost naked stalk and on this a large sperm mass is perched. Compared

with the spermatophore of most other pseudoscorpions and of the scorpions this is extremely simple and probably reduced: clearly however, it could not become less substantial without danger of the semen just sinking into the soil on which it is deposited. Once the spiders had begun the habit of spinning webs, such dangers were overcome for them because a drop of semen will not be absorbed by a strand of spider silk. Since there is evidence (Petrunkevitch, 1952) that the spiders had already developed the web-spinning habit in the Oligocene, there would appear to have been adequate time for the spermatophore to be reduced to nothing because, once there was no further need for it, there would almost certainly be selection against any covering for the sperm mass. It would however be of interest to look carefully at the mating habits of the primitive spiders and especially to see whether amongst the Liphistiomorpha there are animals in which some trace of a membrane persists around the sperm mass and whether this is picked up into the shallow palpal cup as a semi-solid mass rather than as a seminal fluid.

While it is possible that such direct evidence will appear, the hypothesis seems fairly sound on its own merits. It is clear the "double process" is very hard to understand on the basis of an evolution stemming either directly from a Limulus-type external fertilization or indirectly through a stage of ordinary internal fertilization. The present postulate—that of an intercalcated stage of spermatophore deposition and transfer to the female with the aid of the male palps—not only provides a plausible explanation of the origin of this double process, but is in keeping with what we now know of the mating habits of the other arachnid groups.

During the preparation of this paper one of us (A.J.A.) was in receipt of a bursary awarded by the South African Council for Scientific and Industrial Research, to whom our thanks are due.

SUMMARY

1. The essential facts concerning mating behavior in the spiders are outlined together with the main theories of the evolutionary origin of this behavior. It is pointed out that these fail to explain why the spermatic fluid is first deposited before being taken up by the palpal organs of the male.

2. From a comparative survey of the mating habits of those arachnids for which the facts are known, it is suggested that the proto-spiders may have transfered the sperm to the female by way of a spermatophore which they deposited on the substratum. The pedipalps may initially have assisted in this operation or have merely held open the female genital aperture. It is further suggested that the loss of the spermatophore and the retention of the pattern of deposition of the spermatic fluid are to be correlated with the evolution of web-spinning.

LITERATURE CITED

Alexander, A. J., 1956, Mating in scorpions. Nature, Lond., 178. 867–868.
1957, The courtship and mating of the scorpion, *Opisthopthalmus latimanus* Proc. Zool. Soc. Lond., (in the press).

Angermann, H., 1955, Indirekte Spermatophorenübertragung bei *Euscorpius italicus* (Hbst.) (Scorpiones, Chactidae). Naturwissenschaften, 42. 303.

Baerg, W. J., 1928, The life cycle and mating habits of the male tarantula. Quart. Rev. Biol., 3. 109–116.

1954, Regarding the biology of the common Jamaican scorpion. Ann. Ent. Soc. Amer., 47. 272–276.

Bristowe, W. S., 1929, The mating habits of the spiders, with special reference to the problems surrounding sex dimorphism. Proc. Zool. Soc. Lond., 1929. (2) 309–358.

Bristowe, W. S. and Locket, G. H., 1926, The courtship of British Lycosid spiders, and its probable significance. Proc. Zool. Soc. Lond., 1926. (2) 317–347.

Byers, G. W., Lipovsky, J. L. and Kardos, E. H., 1957, J. Parasitol., (in the press).

Comstock, J. H., 1940, The Spider Book. Doubleday, Doran & Co. New York.

Heymons, R., 1901, Biologische Beobachtungen an asiatischen Solifugen. Abhandl. k. Preuss. Ak. wiss. Berlin, 1901. 1–65.

Kew, H. W., 1912, On the pairing of the pseudoscorpions. Proc. Zool. Soc. Lond., 1912. 376–390.

Lankester, E. R., 1881, Limulus an arachnid. Quart. J. Micr. Sci., 21. 504–548. 609–648.

Leone, C. A., 1954, Serological studies of some arachnids, other arthropods, and mollusks. Physiol. Zool., 27. 317–325.

Locket, G. H., 1926, Observations on the mating habits of some web-spinning spiders, with some corroborative notes by W. S. Bristowe. Proc. Zool. Soc. Lond., 1926. 1125–1146.

Menge, A., 1843, Über die Lebensweise der Arachniden. Neueste Schriften Naturf. Ges. Dansig, 4.

Montgomery, T. J., 1903, Studies on the habits of spiders particularly those of the mating period. Proc. Acad. Natur. Sci. Philadelphia. 55. 59–149.

Noble, G. K., 1931, The Biology of the Amphibia. McGraw-Hill, New York.

Nuttall, G. H. F. and Merriman, G., 1911, The process of copulation in *Ornithodorus moubata.* Parasitol., 4. 39–44.

Petrunkevitch, A., 1952, Macroevolution and the fossil record of the Arachnida. Amer. Scient. 40. 99–122.

Piza, S., 1950, Reproductive organs and reproduction in *Tityus babiensis* (Scorpiones-Buthidae). Proc. 8th. Int. Congr. Entomol. Stockholm, 1948. 1026–1027.

Sawaya, P. and Soares, B. A. M., 1949, Reação do caração dos Escorpies do gênero Tityus à desinervação e às drogas coli- e adrenérgicas. Bol. Fac. Filos. Cien. Let. Univ. S. Paulo, 99. 325–333.

Shulov, A., 1957, Proc. 10th. Int. Congr. Entomol. Montreal, (in the press).

Shuster, C. N. Jr., 1950, Observations on the natural history of the American horseshoe crab, *Limulus polyphemus.* Contrib. no. 564. Wood's Hole Oceanographic Inst., 18–23.

Southcott, R. V., 1955, Some observations on the biology, including mating and other behavior, of the Australian scorpion, *Urodacus abruptus* Pocock. Trans. Roy. Soc. Aust., 78. 145–154.

Vachon, M., 1938, Recherches anatomiques et biologiques sur la reproduction et le dévelopment des Pseudoscorpions. Ann. Sci. Nat. Zool., (11) 1–207.

1953. The biology of scorpions. Endeavour, 12, 80–89.

Zolessi, L. C. de, 1956, Observaciones sobre el comportamiento sexual de *Bothriurus bonariensis* (Koch) (Scorpiones, Bothriuridae). Bol. Fac. Agron. Montevideo, Uruguay, 35. 3–10.

THE MAJOR INORGANIC CONSTITUENTS OF ADULT *DROSOPHILA MELANOGASTER**

R. C. KING

Brookhaven National Laboratory, Upton, N. Y. and Northwestern University, Evanston, Ill.

The purpose of this note is to gather together what data are available as to the atomic composition of the adult *Drosophila melanogaster*. Analyses were made at the Brookhaven National Laboratory Chemistry Department for carbon and hydrogen by Miss Nancy Day and for magnesium, sulfur, chlorine and calcium by Dr. R. W. Stoenner. The analyses were of the Oregon-R wild type strain grown on "enriched medium" (King and Wood, 1955) at 25°C. Data are available in the literature for 4 other elements: nitrogen (Charconnet-Harding and Calet, 1951), sodium (Rubinstein, Lwowa and Burlakowa, 1935), phosphorus (King, 1953), and potassium (Kiel, 1943). The oxygen content is calculated by difference. Lithium, copper and manganese are present in trace amounts (King, 1954). The eleven major inorganic constituents of adult *Drosophila melanogaster* (expressed as percent net weight) are: oxygen 69.68, carbon 15.2, hydrogen 9.8, nitrogen 4.2, potassium 0.45, phosphorus 0.31, sulfur 0.15, sodium 0.12, chlorine 0.05, magnesium 0.03, and calcium 0.02. These data are in agreement with the values given for the atomic composition of wet tissue by Lea (1947). The electron density of the fruit fly was calculated, since this value will be useful to radiobiologists. The average number of electrons/mg wet weight is 3.30×10^{20}.

LITERATURE CITED

Charconnet-Harding, F. and C. Calet, 1951, Teneur en trois vitamines du groupe B et en ezote total de *Drosophila melanogaster* en fonction des stades du développement. C. R. Acad. Sci. (Paris) 233: 759–761.

Keil, R., 1943, Der Kaliumgehalt der *Drosophila melanogaster* (Wildform) und die spontane Mutabilität. Biochem. Z. 313: 317–336.

King, R. C., 1953, Studies with radiophosphorus in Drosophila. I. J. Exp. Zool. 122: 541–576.

1954, Dose received by thermal neutron treated *Drosophila melanogaster*. Nucleonics 12: No. 9. 58–9.

King, R. C., and E. M. Wood, 1955, Sex-linked lethal mutations induced by thermal neutrons in male and female *Drosophila melanogaster*. Genetics 40: 494.

Lea, D. E., 1947, Actions of radiations on living cells. Cambridge University Press.

Rubinstein, L. D., Lwowa, W., and Burlakowa, H., 1935, Über den bedarf des tierischen Organismus an Natrium und Calcium (Versuche an Drosophila). Biochem. Z. 278: 418–427.

*Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.

Vol. XCI, No. 860 The American Naturalist September–October, 1957

THE DOMESTIC CHICK: A SUBSTITUTE FOR THE HONEY-GUIDE AS A SYMBIONT WITH CEROLYTIC MICROORGANISMS

HERBERT FRIEDMANN, JEROME KERN, AND JAMES H. RUST*

Smithsonian Institution, Washington, D. C.

The study described in this paper stems from an overall research project on the mechanism of wax-digestion in a group of African birds called honey-guides. In an earlier publication (Friedmann and Kern, 1956 a) detailed accounts of experiments were given which strongly implicated two microorganisms, *i.e.*, a bacterium, subsequently named *Micrococcus cerolyticus* (Friedmann and Kern, 1956 b) and a yeast identified as *Candida albicans*, as the specific agents in the gut of the honey-guide that were responsible for the ability of these birds to maintain themselves for as long as 30 days on a diet of purified wax and water.

During the course of this investigation it became increasingly apparent that the supply of these birds from Africa would be exceedingly limited. Efforts were made therefore to find other animals that might be utilized as substitutes for honey-guides in this study. Mice, rats, and guinea pigs were tried and were found to be inadequate. However, newly hatched domestic chicks, when fed on a mixture of wax and either the Micrococcus or the Candida, or both, were found to digest the wax in much the same manner as the honey-guides.

EXPERIMENTAL FINDINGS

The experimental details were as follows: Varying numbers of new-born domestic chicks were given a diet of finely crushed domestic beeswax mixed with an equal quantity of either *Micrococcus cerolyticus*, *Candida albicans*, or both. The microorganisms were grown in large quantities by procedures described in an earlier paper (Friedmann and Kern, 1956a). In order to insure having active cultures, these were grown daily, as prolonged storage in refrigeration might reduce viability to the point where experimental results might be seriously affected. Each day the combined excreta of all the chicks were collected and weighed, the wax extracted with chloroform and the saponification numbers of the extracted wax determined. It may be explained, at this point, that the saponification number of a waxy material is an accurate index of the degree to which the substance has been broken down into simpler components. The saponification number is the number of grams of KOH required to hydrolyze one gram of wax, and it varies inversely with the molecular weights of the substances present. In other words, the saponification value or number of the waxy material rises

*Published by permission of the Secretary of the Smithsonian Institution

as the decomposition advances breaking down the substance into simpler and simpler components, and it remains constant if no alteration occurs.

The results of a typical experiment are graphically presented herewith (fig. 1). The control line is derived from chicks that were fed crushed wax by itself, without either the Micrococcus or the Candida. It appears, from

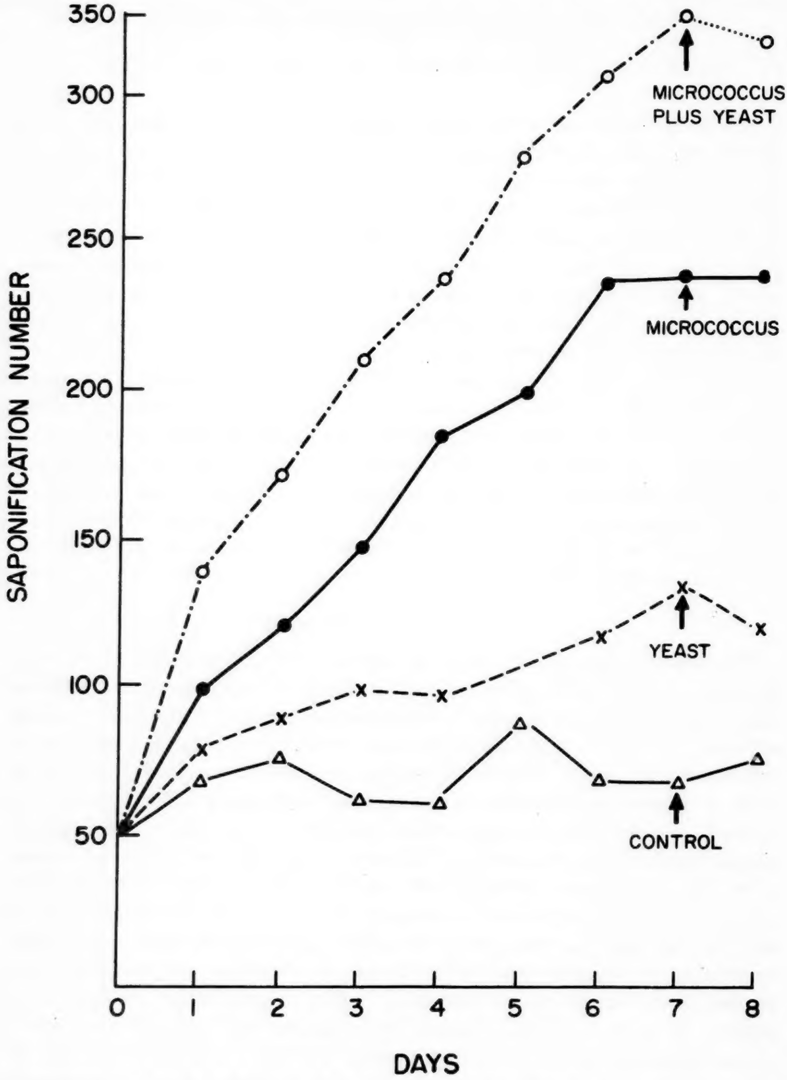

FIGURE 1. Relation between digestion (indicated by rise in saponification number) and presence of yeast and/or a bacterium in beeswax fed to newly hatched chicks.

the data presented, that the chick, by itself, does not demonstrably alter the composition of the wax although considerable quantities of it were consumed. However, when the wax was supplied together with either or both of the cerolytic microbes, a significant rise in the saponification number was noted in the wax recovered from the excreta. The change was greater with the bacterium than with the yeast. None of the chicks kept on a solely wax and water diet survived for more than 7 or 8 days. Data were also obtained on the change of saponification number of wax after it had been mixed with the microorganisms but without exposure to the chicks. In each case the results were negative or, at best, inconclusive. That is to say, when approximately equal quantities of wax and of microorganisms were mixed, incubated from 24 hours to 7 days, the wax then reextracted and saponification numbers determined, there was little or no change in the latter even though both microbes exhibit cerolytic activity when tested by more sensitive methods.

The catabolism of myricyl palmitate, a major constituent of most waxes, can also be followed and measured by an increase in its saponification number. When a mixture of myricyl palmitate and the cerolytic microorganisms was fed to chicks, the results were similar to those shown for whole wax in our graph.

DISCUSSION

In the course of the work the chicks never survived for as long as did the honey-guides in our earlier studies. Whether this is due to wax being a less satisfactory partial diet to a growing young bird (chick) than to an adult (honey-guide) is not known, nor is it known whether the Micrococcus or the Candida succeed in becoming established as a permanent part of the intestinal microflora of the chick. It is quite probable that they do not, but we have no real proof of this. While the chick—Micrococcus, or the chick—Candida relationship is an artifical, experimentally induced one, it is not certain just how different it may be from what occurs in a state of nature in the African honey-guide. All examples of the latter species examined (and the number was more than sufficient to establish the point) invariably had the microbes present, as well as other microorganisms that showed no cerolytic activity. We have ascertained that the birds acquire the bacterium and the yeast from the wild bee-comb, on which we have found them to occur. It follows from this, that, under natural conditions, the honey-guides are actually constantly reinoculating their alimentary tracts with the wax-breaking microbes every time they ingest bee-comb. It is not known how long one inoculation might survive in these birds, although earlier studies showed (Friedmann and Kern, 1956 a) that captive honey-guides fed on pure beeswax and water were able to survive for periods up to 30 days, which suggests a greater viability of the cerolytic microbes in the honey-guide intestine than is probable in that of the chick.

Inasmuch as the question is raised above as to whether the partial diet contained in wax may be less satisfactory to young chicks than to adult birds (honey-guides), it may be mentioned that honey-guides eat no wax,

as far as known, until they are full grown. However, the conditioning factors here are different. The honey-guides are parasitic in their breeding, leaving their eggs in nests of other birds—bee-eaters, barbets, woodpeckers, sparrows, etc., none of which feed their young (or their parasitic foster-young) any wax. The honey-guides grow to full size in the nests of their brood hosts, but when they are once on their own they clearly have the habit of feeding on the contents of wild bees' nests, and, from this source they derive primarily the wax of the comb.

It still remains to be established whether the Micrococcus actually fits all the requirements of a true symbiotic bacterium as far as the honey-guides are concerned, or if it is more in the nature of a useful coincidence, but it seems to be essential for that part of the birds' nutrition that is derived from ingested wax. It is possible that the arrangement may be favorable to the Micrococcus as well as to the bird; at least it is in the intestinal environment of the latter that the microbe's cerolytic metabolism is activated.

On the whole, in such cases of endosymbiosis, there is usually a marked degree of specificity involved, the body of a particular species, or related group of species, of hosts only being suitable for each species of microorganism, and, conversely, each particular species of microbe being suitable for, or adapted to, a very limited range of hosts. As Caullery (1952, p. 222) observes, in his brief discussion on intestinal bacteria, "... the precise interpretation of the role of these symbionts in the physiology of the organism which houses them presupposes an extremely thorough knowledge of the hosts' metabolism which, in general, we are far from possessing..." It is, thus, all the more interesting to find that hosts as different as young domestic chicks and honey-guides are both able to effect a working, temporary, symbiosis with the wax-splitting Micrococcus and Candida. This is quite apart from the fortunate adaptability of the chick as a "substitute honey-guide" which permitted our studies to proceed without having to depend on an insufficient supply of the normal host.

Symbiotic partnerships ordinarily exist because either or both of the organisms involved exhibit some specialized behavior or activity whose function appears to be intimately connected with the inception and maintainance of the symbiotic association. Davenport (1955, pp. 44–45) has used the name "symbiosis-effective" for such stimuli as elicit behavior serving to effect and to maintain such cooperative partnerships. This concept applies, however, to organisms higher in the behavior scale than microbes, as it is difficult to see any demonstrable effective stimuli between a bacterium and a bird. It is probably because of this absence of specific stimuli on a behavioristic level that it is possible for one species, the domestic chick, to replace, even at a lesser degree of effectiveness, the usual symbiont, the honey-guide.

The results reported in this paper, together with other data discussed above, suggest an interesting and still more involved biological situation. The observation that neither the chick nor the microorganism is able significantly to digest the wax without the other indicates that a symbiotic

relationship exists between them, wherein each contributes a necessary element. This implies that the avian donation to this symbiosis is not necessarily specific, although the substance or substances produced by the honey-guide may be more effective than those given off by the chick. It is possible that our success with the chick as a substitute for the honey-guide may imply that the environment of the former's gut may be all that is necessary for this symbiotic relationship to exist. Experiments are under way to determine precisely what factors from the bird and from the microorganism make this mutual reciprocal relationship possible, not only in the normal symbiont, the honey-guide, but also in the substitute for it, the the young domestic chick.

Another aspect of the data here presented may be stressed. In earlier work on the mechanism of wax digestion by the honey-guide much of the evidence as to the role played by the Micrococcus and the Candida was of an indirect nature (Friedmann and Kern, 1956 a). These microorganisms were indeed implicated in the process, but at that time no direct evidence had been obtained. It now appears, from the fact that a non-wax-digester such as the newly hatched domestic chick, when given these microbes with ingested wax is then able to break down the wax, that these organisms are the effective wax digesters, provided the proper environment is made available for them. The intestine of the bird (chick or honey-guide) seems to provide the favorable conditions for these cerolytic microbes.

SUMMARY

The experiments described in this report represent a portion of a more comprehensive study which deals with the mechanisms of wax digestion in an African bird, the lesser honey-guide, *Indicator minor*. It has been found that newly hatched domestic chicks will also metabolize waxes provided these materials are fed to them together with cerolytic microorganisms initially isolated from the gut of the honey-guide. The chicken alone is not able to break down the wax, and the microorganisms alone can do far less along this line than when in the intestinal environment of the host bird.

ACKNOWLEDGMENTS

The authors express their gratitude to Dr. S. J. Ajl for his suggestions and advice in the course of the work. This study was done, as part of a larger program, with support from the John Simon Guggenheim Memorial Foundation.

LITERATURE CITED

Caullery, Maurice, 1952, Parasitism and symbiosis. 340 pp.

Davenport, Demarest, 1955, Specificity and behavior in symbioses. Quart. Rev. Biol., 30:29–46.

Friedmann, Herbert and Kern, Jerome, 1956 a, The problem of cerophagy or wax-eating in the honey-guides. Quart. Rev. Biol., 31:19–30

1956 b, *Micrococcus cerolyticus*, nov. sp., an aerobic lipolytic organism isolated from the African honey-guide. Canad. Journ. Microbiol., 2:515–517.

LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

"SEX-RATIO" IN *DROSOPHILA PROSALTANS*—A CHARACTER DUE TO INTERACTION BETWEEN NUCLEAR GENES AND CYTOPLASMIC FACTORS.

One of the fundamental problems of physiological genetics concerns the interrelations between nuclear genes and cytoplasmic factors in development and evolution. The present paper offers a new opportunity for studies in this field. It reports an interesting case of "sex-ratio" in *Drosophila prosaltans* involving an interaction between nuclear genes and plasmagenes.

Production of unisexual progenies or significant deviations from the normal 1:1 sex ratio, almost always in favor of females, are known in several species of Drosophila. In most instances so far studied the genetic basis of these phenomena consisted of genes, or groups of genes, located in the X chromosomes; males which carry such "sex-ratio" genes produce mostly or exclusively daughters, regardless of the genotype of the females to which they are mated. The "sex-ratio" in *Drosophila prosaltans* to be described below is, on the contrary, inherited through the females only, in such a manner that females of certain strains produce only, or mostly daughters. However, the unusual interest of this "sex-ratio" case is that it is the only one thus far known in Drosophila which is controlled both by maternally transmitted cytoplasmic factors or "plasmagenes" and modified by nuclear genes transmitted through both sexes. Owing to this, the situation in *Drosophila prosaltans* presents a remarkable parallel to that discovered in Paramecium by Sonneborn (1950, 1954, and other works). Indeed, the inheritance of the "killer" character in Paramecium and of the "sex-ratio" in *Drosophila prosaltans* are closely similar.

The discovery of the sex-ratio character in *D. prosaltans* was made accidentally by the senior author (Cavalcanti, 1950) in the course of an experiment analyzing the frequency of lethal genes in natural populations. The character was interpreted originally as being due exclusively to cytoplasmic factors, maternally inherited (Cavalcanti and Falcão, 1954). We have now gathered strong evidence that we are dealing with a more complex situation, due to an interaction between nuclear genes and cytoplasmic factors.

The experimental data will be presented in full elsewhere. In the present note we shall describe only the working hypothesis which we have adopted and the crucial evidence that supports it. In our interpretation, the charac-

ter "sex-ratio" in *D. prosaltans* is due to interaction between nuclea genes and cytoplasmic factors which can be denoted as follows:

Sr...........the "sex-ratio" gene for progenies consisting of mostly or only females.

sr...........the recessive allele permitting the appearance of males.

o (omikron)—the cytoplasmic factor necessary for realization of the "sex-ratio" condition.

The experimental evidence can be summarized under the following eight headings:

1. The sex-ratio females carry the omikron factor in their cytoplasms. The absence of males in the progenies of such females is due to the death of the XY eggs with omikron plasmon.

2. The non-sex-ratio or normal flies (females and males) do not carry the omikron plasmagenes.

3. The plasmagene is transmitted exclusively through the eggs of the sex-ratio females and it is not contagious under normal biological conditions. We do not know as yet if it can be experimentally transmitted by injection or by organ transplantation.

4. The reproduction of the plasmagene occurs only in flies which carry the *Sr* gene in homozygous or in heterozygous condition. In recessive homozygotes *sr/sr* the plasmagene is not retained.

5. The only known manifestation of the *Sr* gene is to permit the reproduction of the plasmagene omikron. This gene does not have any perceptible effects either on the sex-ratio or on any other traits of the flies, so that *Sr/Sr, Sr/sr* and *sr/sr* individuals are indistinguishable phenotypically.

6. Normal females and males never give rise to sex-ratio progenies.

7. The sex-ratio females occasionally give origin to normal females evidently without the plasmagene, when crossed to suitable males.

8. The males do not transmit the plasmagene at all. They function only as nuclear gene donors.

Two kinds of sex-ratio females can be distinguished by their breeding behavior, those of the genotype *Sr/Sr* with omikron, and those *Sr/sr* with omikron. Normal flies, females or males, have the genotypes *Sr/Sr, Sr/sr,* or *sr/sr,* the females lacking the plasmagene omikron. Natural populations of *Drosophila prosaltans* in Brazil are mixtures of flies of all these genotypes. For experimental purposes we have established, by systematic pair matings and suitable testing, two kinds of analyzer stocks, namely (a) the "sex-ratio maintainer stock," homozygous for the *Sr/Sr* genes, and (b) the "sex-ratio disrupter stock," homozygous for the *sr/sr* genes. With the aid of these stocks, the above working hypothesis may be tested as follows. Females collected in nature are placed in single cultures and allowed to produce progenies. Some of them give progenies consisting exclusively or predominantly of daughters. These daughters are crossed and backcrossed to males of the two analyzer stocks. The crosses to *SR/SR* males produce mostly or only female progenies. The crosses to *sr/sr* males give also mostly or only females in the F_1 generation, but some cultures with normal ratios of females and males in the backcrosses to the sex-ratio disrupter.

By means of such tests we analyzed 9 sex-ratio strains derived from sex-ratio females collected in the states of Ceara and Maranhão, in northern Brazil. From 241 individual crosses of sex-ratio females with males of the "sex-ratio maintainer stock" we obtained in three consecutive generations 9,747 females and no males. With the "sex-ratio disrupter stock" we obtained in the F_1, in 44 individual crosses, 2,067 females and 5 males. In the backcrosses, as expected, progenies with approximately equal numbers of females and males were obtained.

It seems worth while to emphasize the remarkable parallel between the mechanism thus found in Drosophila and the remarkable discovery of Sonneborn of the "killer" character in Paramecium. The two groups described in *Drosophila prosaltans,* the sex-ratio females with plasmagenes and normal females and males without plasmagenes, are close analogues of what happens in various stocks of *Paramecium aurelia* "variety 4." The stock 51 of the infusorium carries the kappa cytoplasmic factor and the gene *K*, and thus corresponds to our sex-ratio females with the plasmagene omikron and the gene *Sr* (required for the maintainance of omikron). The stock 47 of Paramecium which has the gene *K* but no kappa, corresponds to our "sex-ratio maintainer stock," with *Sr* genes but without the plasmagenes. Finally the stocks 29 or 32 of Paramecium which have no kappa and also do not have the gene *K* (having *k* instead) are paralleled by our "sex-ratio disrupter stocks" with no plasmagene omikron and also without the gene *Sr,* being homozygous for the recessive allele *sr.* All the Paramecium stocks above mentioned belong to one species, and so do the sex-ratio and normal strains of *D. prosaltans.* A further parallel concerns the physiological action in both cases. In Paramecium as well as in Drosophila there is a lethal effect which kills sensitive individuals of Paramecium, while in Drosophila it kills the XY zygotes, or sensitive zygotes if we wish so to call them.

REFERENCES

Cavalcanti, A. G. L., 1950, Contribuição a genetica dos populações naturais: análise dos gens autossomicos recessivos dos cromossomos II e III de *Drosophila prosaltans* Duda. Dept. Imprensa Nacional. Rio de Janeiro. Brazil.

Cavalcanti, A. G. L., and D. N. Falcão, 1954, A new type of sex-ratio in *Drosophila prosaltans* Duda. Proc. 9th Int. Congr. Genetics. Part II: 1233-1235.

Sonneborn, T. M., 1950, The cytoplasm in heredity. Heredity 4:11-36.

1954, Patterns of nucleocytoplasmic integration in Paramecium. Proc. 9th Int. Congr. Genetics: 307-325.

A. G. LAGDEN CAVALCANTI
D. N. FALCÃO
L. E. CASTRO

CENTRO DE PESQUISAS DE GENETICA
UNIVERSIDADE DO BRASIL
RIO DE JANEIRO
May 17, 1957

ON THE ROLE OF OMEGA BACTERIOPHAGE IN FORMATION OF CROWN-GALL TUMOR CELLS*

Beardsley[1] recently discovered that *Agrobacterium tumefaciens*, strain B_6, here designated B_6 (Ω), is lysogenic. He also performed preliminary experiments suggesting a causal relationship between the bacteriophage, omega, produced by this strain and the induction of crown-gall tumor cells. It seemed possible that the bacteriophage could act directly on susceptible plant cells as an essential agent in tumor-cell formation, being itself the tumor-inducing principle (T-iP) synthesized by virulent bacteria,[2] or acting in conjunction with T-iP.

Investigation of the proliferations that were reported has shown that they failed to grow on a medium which supports the growth of crown-gall tissues. Critical examination of the techniques employed indicated that these overgrowths were not phloem as initially reported but were cambial in origin and thus capable of extensive non-tumorous proliferation. Ultraviolet-induced lysates of B_6 (Ω) containing ca. 10^{10} phage per ml. were incapable of inducing tumor-cell formation in carrot phloem tissues under conditions where T-iP did induce crown-gall.[3] The activity of T-iP was destroyed by deoxyribonuclease[3,4] whereas this enzyme was inactive in destroying the infectivity of omega bacteriophage against susceptible bacteria.[5] The identification of omega bacteriophage with T-iP appears untenable.

The possibilities that T-iP is heterogeneous, consisting of both omega bacteriophage and a DNase-sensitive fraction, or that omega or its prophage is required for the synthesis of T-iP appear unwarranted in view of the following finding. The virulence of B_6 (Ω) was compared by quantitative bioassay,[6] with that of three of its derivitives: 1) B_6, a phage-sensitive strain, 2) B_6-1 (Ω), a lysogenic strain derived from B_6 by reductive infection, and 3) B_6-1, a strain sensitive to omega derived from B_6-(Ω) by heavy ultraviolet irradiation. All tested strains were equally virulent. If lack of immunity to omega bacteriophage by B_6 and B_6-1 is presumptive evidence for the absence of omega prophage in these strains,[7] this finding is an indication that omega is probably not required for the activity or the synthesis of T-iP.

LITERATURE CITED

[1]Beardsley, R. E., 1955, Phage production by crown-gall bacteria and the formation of plant tumors. Amer. Nat. 89: 175–176.

[2]Braun, A. C., 1947, Recent advances in the physiology of tumor formation in the crown-gall disease of plants. Growth 9: 325–337.

[3]Klein, R. M., and J. L. Knupp, Jr., 1957, Sterile induction of crown-gall tumors on carrot tissues in vitro. Proc. Nat'l. Acad. Sci. 43:

[4]Manigault, P., A. Comandon, and P. Slizewicz, 1956, Preparation d'un "principe inducteur" de la tumeur du *Pelargonium*. Ann. Inst. Pasteur 91: 114–117.

[5]Beardsley, R. E., 1957, Ph.D. Thesis, Department of Zoology, Columbia University.

*This work was supported by a grant from the American Cancer Society recommended by the Committee on Growth of the National Research Council, and by a grant from the Damon Runyon Memorial Fund for Cancer Research. The advice of Prof. F. J. Ryan is gratefully acknowledged.

[6]Klein, R. M., and I. L. Tenenbaum, 1954, A quantitative bioassay for crown-gall tumor formation. Amer. J. Bot. 42: 709–712.
[7]Lwoff, A., 1953, Lysogeny. Bact. Rev. 17: 269–337.

NEW YORK BOTANICAL GARDEN,
BRONX PARK, NEW YORK
DEPARTMENT OF BIOLOGY,
MANHATTAN COLLEGE, NEW YORK

RICHARD M. KLEIN
ROBERT E. BEARDSLEY

June 12, 1957

THE FEASIBILITY OF INVESTIGATING "GENETIC FINE STRUCTURE" IN HIGHER PLANTS

The recent important investigations by Benzer (1957) in bacteriophage may force a complete revision of our classical concept of the gene as a functional entity within which genetical recombination does not take place. It is of paramount importance for genetics that we know if the results obtained with phage are indicative of unique recombination procedures for such organisms. Alternatively the intralocus recombination of genetic material may be common to all organisms but occurring at such a low rate of frequency that it escapes our notice in progenies of the sizes to which we are limited in genetical investigations with higher organisms. Even making exhaustive efforts to deal with large numbers may not be sufficient as shown by Green (1956) for Drosophila.

If we are to resolve the question, radical changes in technique are necessary. One possibility is to deal with the genetics of the gametophytic generation of higher plants rather than with the sporophytic generation. Obviously the loci which can be used in investigations of this type are limited in number, but they do exist. Demerec (1924) and Brink and MacGillivray (1924) showed that *waxy* pollen grains in maize stain brown when treated with a mixture of iodine and potassium iodide in alcohol in contrast to normal pollen grains which stain a deep blue. These staining reactions indicate the type of starch contained in the pollen grains. *Waxy* starch is exclusively amylopectin while normal starch consists of a mixture of approximately 75% amylopectin and 25% amylose. It is the amylose constituent of normal starch which is responsible for the blue color with the iodine stain. The starch type of a pollen grain is governed by its own genetic constitution and not by the genotype of the plant which produces it. Further, a maize plant produces millions of pollen grains and in a conveniently packaged form which can be preserved almost indefinitely. A number of independent mutations at the *waxy* locus have been reported and are available for study.

Using a Virtis homogenizer it is possible to break up a number of anthers and release undamaged pollen grains. One to two hundred thousand pollen

grains in a gelatin mounting medium can then be spread on a slide under a large cover slip. Using a rather weak iodine stain, one blue-staining pollen grain per slide can be readily distinguished among the brown-staining grains. Our current investigations are concerned with the mutation rate in the *waxy* stocks which have been collected. This information is essential since it indicates the background against which one must consider the detection of possible low levels of recombination. At the same time, intercrosses between many of these stocks have been made.

If any of these independent mutations at the *waxy* locus represent changes at different mutational sites within the locus and if intralocus recombination takes place, it should be detectable in the gametes produced by plants which are intercrosses between those stocks representing different mutational sites. When recombination takes place, it should reconstitute a functional unit at the *waxy* locus; in this case some amylose will be formed, and the pollen grain will stain blue. Where the rate of natural mutation is low (and our data indicate that this is so for all stocks investigated), any significant increase above this rate will be indicative of recombination within the locus. If it occurs, then a systematic program of all possible intercrosses between independent mutations at the locus should allow the construction of a progressively more detailed map of the locus.

In this particular instance, both advantages and disadvantages can be foreseen. On the disadvantageous side the pollen grains are killed so that it is not possible to test further the supposed product of recombination. On the other side, data obtained are not complicated by successive rounds of mating as with phage. The *cis-trans* test is easily made in the intercrosses between stocks. Further, it may be possible by biochemical or immunological techniques to find what each independent mutation involves in terms of inability to support the formation of amylose. Apart from the above considerations, it should be possible with a program as detailed, to obtain data which will resolve the paradox with which we are confronted in genetics at the present time.

LITERATURE CITED

Benzer, Seymour, 1957, The elementary units of heredity. A Symposium on the Chemical Basis of Heredity. Johns Hopkins Press. Baltimore.

Brink, R. A. and J. H. MacGillivray, 1924, Segregation for the *waxy* character in maize pollen and differential development of the male gametophyte. Amer. Jour. Bot. 11:465–469.

Demerec, M., 1924, A case of pollen dimorphism in maize. Amer. Jour. Bot. 11:461–464.

Green, M. M., 1956, A further analysis of the forked locus in *Drosophila melanogaster.* Proc. Nat. Acad. Sci. 42:73–77.

OLIVER E. NELSON, JR.

DEPARTMENT OF BOTANY AND PLANT PATHOLOGY
PURDUE UNIVERSITY, INDIANA
August 2, 1957

COPULATORY FUNCTION OF THE MODIFIED PECTORAL FIN OF GAMBUSIIN FISHES[1]

Sexual dimorphism of fin structure has been noted in many fishes. In many, a fin, usually the anal, is used for transmission of sperm during copulation. Among the best known examples are the members of the family Poeciliidae (Rosen and Gordon, 1953). These fishes develop sexual dimorphism of other fins. The dorsal and caudal fin rays are elongated and the membranes highly colored in males. Sexual dimorphism of the paired fins is less noticeable as it is seldom associated with brilliant coloration. Nevertheless, as Clark, Aronson, and Gordon (1954: 166) clearly pointed out for the pelvic fin, the morphologic dimorphism is closley associated with courtship.

Turner (1942) first described sexual dimorphism in the thickness of the pectoral fin rays of the common mosquitofish, *Gambusia affinis* (Baird and Girard). He reported that Carl L. Hubbs had found the dimorphism to occur in all species of Gambusia, in Flexipenis and Heterophallus, and to a slight extent in Belonesox. Turner did not discuss the probable function of the modification. Hubbs and Hubbs (1945) pointed out the bilateral asymmetry in the right and left halves of the modified pectoral fin rays of adult males of Gambusia. Carl L. Hubbs (1950) discussed the highly modified right pectoral fins of *Xenodexia ctenolepis* Hubbs. He suggested that the pectoral fin may hold the anal fin (gonopodium) of the male during copulation. In the absence of living stocks he could not confirm this hypothesis. Clark Hubbs (1957) discussed the sexual dimorphism of the pectoral fin of *Gambusia heterochir* Hubbs. Sexual dimorphism of the pectoral fin in this species differs from that of other mosquitofishes in being more extreme and in the development of a distinct notch on the upper margin. He postulated that the notch may be used to steady the gonopodium during copulation. Warburton, *et al.* (in press) reported many observations of this action and thus confirmed the postulate.

Turner (1942) pointed out that thickened upper pectoral rays occur in males of four genera of poeciliids, comprising the entire tribe Gambusiini (Carl L. Hubbs, 1926). Both Carl L. Hubbs and Clark Hubbs have since looked at the pectorals of other species of Gambusia and found that all species examined have some sexual dimorphism. Only *G. heterochir* has the extreme modification. As the modified pectoral fin is used during copulation in *G. heterochir,* it is of interest to determine whether the less extremely modified pectorals of related fishes are likewise of copulatory use.

Males of three species, *Gambusia affinis, G. geiseri* Hubbs and Hubbs, and *G. hurtadoi* Hubbs and Springer, have been isolated in small observation chambers for at least 24 hours. Females were then introduced and courtships observed for as long as 30 minutes. We find, as did Clark,

[1]This work is supported by Research Grant NSF-2214 from the National Science Foundation. The manuscript has been read and criticized by Drs. W. F. Blair and Carl L. Hubbs.

Aronson, and Gordon (1954), that this technique increases the sexual activity of males and facilitates observations. Males of all three species repeatedly place the gonopodium over the pectoral fin during courtship. This act is essentially identical to that of *G. heterochir* described by Warburton, *et al.* (in press). The appropriate pectoral is held rigidly at about 90° to the body axis and the gonopodium placed over its upper margin. The body often is curved toward the appropriate pectoral fin. Males of the four species and hybrids between *G. affinis* and *G. heterochir* are all ambidextrous. Not only is the gonopodium so placed during copulations, but also during thrusts. On each occasion where observation was possible the pectoral was used to support the gonopodium in copulation of all species of Gambusia. However, observations of other species (especially distantly related forms such as *G. panuco* Hubbs, *G. nicaraguensis* Günther, and *G. lemaitrei* Fowler) have not been carried out because living stocks are not available in our laboratory. However, as the pectoral fin structure is essentially similar, use of the structure is likely to be the same.

Pectoral support of the gonopodium during copulation appears to be typical of members of the genus Gambusia and thus may be expected to have occurred in the stem species. One species, *G. heterochir,* has a highly specialized pectoral with a notch in the upper margin to further support the gonopodium. Therefore, the extreme pectoral modification must have followed the behavioral activity. The only other hypotheses—parallel development of the behavioral activity or loss of the pectoral notch in all other species—are far less likely. We believe that the pectoral notch is of selective advantage in that it gives ventral and lateral stability to the gonopodium during copulation. The slightly modified pectoral of other mosquitofishes merely gives ventral stability.

SUMMARY

Extreme modification of the pectoral fin to support the gonopodium during copulation occurs in *Gambusia heterochir.* As males of all species we have examined in this genus support the gonopodium with the pectoral fin, evolution of the structural change most likely follows that of the behavior.

LITERATURE CITED

Clark, Eugenie, Lester R. Aronson, and Myron Gordon, 1954, Mating behavior patterns in two sympatric species of xiphophorin fishes: Their inheritance and significance in sexual selection. Bull. Am. Mus. Nat. Hist., 103: 135-226, pls. 1-9.

Hubbs, Carl L., 1926, Studies of the fishes of the order Cyprinodontes. VI. Material for a revision of American genera and species. Misc. Pub. Mus. Zool. Univ. Mich., 16: 1-87, pls. 1-4.

1950, Studies of cyprinodont fishes. XX. A new subfamily from Guatemala, with ctenoid scales and a unilateral pectoral clasper. Misc. Pub. Mus. Zool. Univ. Mich., 78: 1-28, pls. 1-4.

Hubbs, Carl L., and Laura C. Hubbs, 1945, Bilateral asymmetry and bilateral variation in fishes. Pap. Mich. Acad., 30 (1944): 229-310, pl. 1.

Hubbs, Clark, 1957, A new poeciliid fish from Texas, with an account of its hybridization with *G. affinis.* Tulane Studies Zool., 5: 1-16.

Rosen, Donn E., and Myron Gordon, 1953, Functional anatomy and evolution of male genitalia in poeciliid fishes. Zoologica, 38: 1-47, pls. 1-4.

Turner, C. L., 1942, Sexual dimorphism in the pectoral fin of Gambusia and the induction of the male character in the female by androgenic hormones. Biol. Bull., 83: 389–400.

Warburton, Barbara, Clark Hubbs, and Donald W. Hagen, (in press) Reproductive behavior of *Gambusia heterochir.* Copeia.

CLARK HUBBS
ROBERT A. REYNOLDS

THE UNIVERSITY OF TEXAS
AUSTIN, TEXAS
August 30, 1957